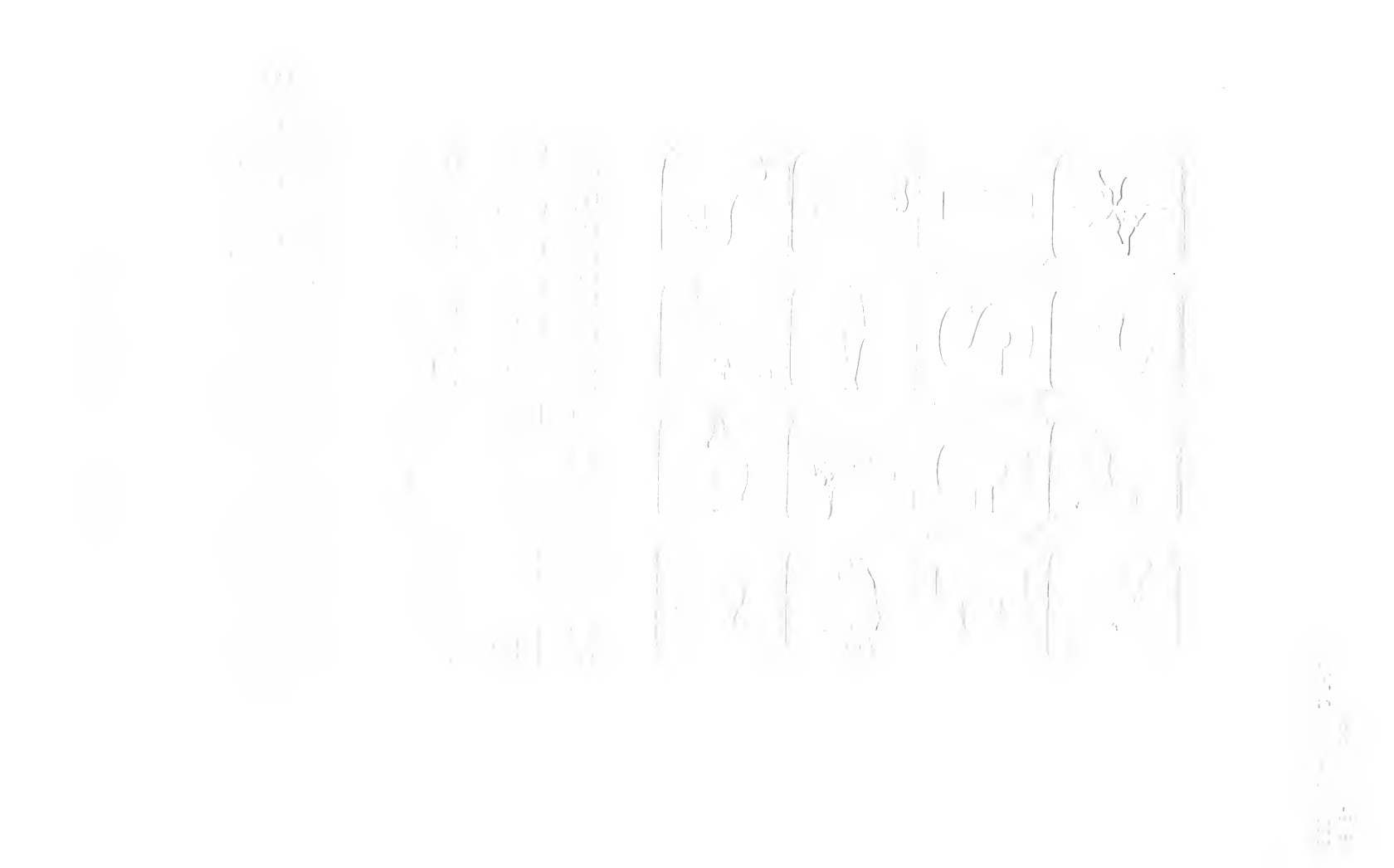

INTERNATIONAL JOURNAL OF COMPARATIVE PSYCHOLOGY

Volume 2, Number 4

ARTICLES

THE INTERNATIONAL JOURNAL OF COMPARATIVE PSYCHOLOGY is co-sponsored by the International Society for Comparative Psychology, an affiliate of the International Union of Psychological Sciences and the University of Calabria, Italy. In consonance with the goals and activities of the Society, it publishes reports of studies in the evolution and development of behavior of all species; on the use of the comparative method for the understanding of behavioral processes; and the examination of the adequacy of psychological and evolutionary theories. It accepts articles that deal with historical and contemporary relationships between humans and other animals; that elucidate problems of ecological and behavioral processes in resource management; that explicate fundamental concepts about human evolution; and that present research results and develop theories about the development and evolution of behavior. Articles on the contemporary and historical organization of knowledge about comparative psychology; promoting public education on the evolution and development of behavior; and about the training of students in comparative psychology are also invited. Book reviews; letters discussing published articles with replies by authors; notices of new books, journals, meetings and competitions or other matters of interest to the readers will be published at the discretion of the editors. The Editor and Co-Editor are elected by the Operations Committee of the Society. National Editors are appointed by the Editors and the Operations Committee.

MANUSCRIPTS should be submitted in triplicate to the National Editor, if there is none, the three copies should be sent to Ethel Tobach, Editor. See inside back cover for style requirements.

SUBSCRIPTIONS are on an annual basis: $75.00 per volume of institutions; $32.00 for individuals; handling and postage costs to be paid by individuals outside the USA; members of the International Society for Comparative Psychology receive their yearly subscription at the rate of $24.00 (sliding scale) as part of their membership dues. For membership information, write to Dr. Jeanette Ward, Department of Psychology, Memphis State University, Memphis, TN 38152, USA.

ADVERTISING inquiries should be addressed to the Advertising Sales Representative, Daniel S. Lipner, Weston Media Associates, P.O. Box 1110, Greens Farms, Connecticut 06436—telephone: (203) 261-2500.

 Published quarterly in the Fall, Winter, Spring, and Summer. The International Journal of Comparative Psychology is a trademark of Human Sciences Press, Inc. Second-class postage pending at New York, NY and additional mailing offices. The address for the journal is: The International Journal of Comparative Psychology, 233 Spring Street, New York, NY 10013-1578. **Postmaster:** Send changes of address and return journals if undeliverable to: International Journal of Comparative Psychology, Human Sciences Press, 233 Spring Street, New York, NY 10013-1578. Please return entire journal—do not remove cover.

ISSN 0889-3667 IJCPE82(4)211-264(1989)

The International Journal of Comparative Psychology, Vol. 2, No. 4, Summer 1989

PHYSIOLOGICAL ROLES OF NERVE GROWTH FACTOR IN ADULT RODENTS: A BIOBEHAVIORAL PERSPECTIVE

Enrico Alleva
Istituto Superiore di Sanita

Luigi Aloe
Istituto di Neurobiologia, CNR

ABSTRACT: The present review is concerned with the biological role(s) exerted by Growth Factor (GF) protein molecules in adult rodents. In fact, despite the increasing amount of papers published in the last two-three decades about the physiological roles played by Nerve GF and Epidermal GF (as well as by related polypeptide molecules) on the ontogenesis of rodent peripheral and central nervous systems, very little attention has been given to adult regulations involving these two factors. We here report about our studies concerning the biological significance of the huge quantity of NGF stored in the submaxillary salivary glands of the adult male mouse. When released into the bloodstream as a result of psychosocial stress, salivary NGF affects peripheral nervous structures (chromaffine cells and ganglia) and peritoneal mast-cells. Following psychosocial stress, NGF production is enhanced in specific hypothalamic zones. Adult regulations regarding the concomitant EGF release from salivaries are also discussed.

RIASSUNTO: La presente rassegna è centrata sul ruolo esercitato da fattori di crescita polipeptidici (NGF ed EGF) sulle regolazioni fisiologiche di roditori nello stadio adulto. Infatti, nonostante la mole crescente di lavori pubblicati negli scorsi tre decenni sul ruolo biologico esercitato da tali fattori sull'ontogenesi dei sistemi nervosi periferico e centrale di roditori, poca o nulla attenzione e stata invece prestata riguardo al ruolo esercitato da NGF ed EGF in regolazioni fisiologiche nell'adulto. Vengono riportati i risultati di una serie di studi condotti dagli Autori sul rilascio del NGF accumulato in elevatissime quantità all'interno delle ghiandole sottomascellari del topo maschio adulto, e che viene rilasciato in circolo esclusivamente in seguito a stress di tipo psicosociale. Tale rilascio causa ipertrofia di vari tessuti surrenalici, attiva i gangli periferici, e causa una selettiva degranulazione in elementi mastocitari. Lo stress psicosociale eleva nel contempo la produzione di NGF in particolari aree ipotalamice. Vengono infine discussi anche dati riguardanti il ruolo biologico dell'EGF nel roditore adulto, proteina anch'essa immagazzinata nelle sotto-mascellari murine e rilasciata in circolo in seguito a stress psicosociale.

Address correspondence to: Dr. Enrico Alleva, Section of Behavioral Pathophysiology, Laboratorio Fisiopatologia O.S., Istituto Superiore di Sanita, viale Regina Elena, 299 I-00161 Rome, Italy.

INTRODUCTION

Nerve Growth Factor (NGF) is a neurotrophic factor with a well established physiological role in promoting and/or maintaining survival of specific portions of the peripheral and central nervous systems (Levi-Montalcini & Angeletti, 1968; Calissano, Cattaneo, Aloe, and Levi-Montalcini, 1984; Black, 1986; Levi-Montalcini, 1987; Thoenen et al., 1987). NGF exerts trophic, tropic, and differentiative effects on specific cellular targets, and mitogenic properties on developing chromaffine cells (Zaimis, 1971; Aloe and Levi-Montalcini, 1979; Lillien and Claude, 1985). In addition, evidence accumulated over the past few years shows that NGF is capable of exerting specific effects on basal forebrain cholinergic neurons of the CNS (Gnahn et al., 1983; Seiler and Schwab, 1984; Korshing, 1986; Shelton and Reichardt, 1986), opening new perspectives about its role in biobehavioral studies.

Although the physiological role of NGF in living organisms is still a matter of great debate, what we would like to stress here is that most of the current debate is centered on the developmental properties of NGF, and that very little room has been left for speculations about NGF (or other well-known GFs) roles on adult CNS/behavioral regulations. For example, Epidermal Growth Factor (EGF), which was considered almost exclusively a GF specific for epidermal-derived tissues has been recently proved to have several target tissues at the CNS level (Fallon et al., 1984, Gomez-Pinilla, 1988; Pioro and Cuello, 1988; Werner et al, 1988). Such a picture is now changing, since, e.g., review articles on NGF effects at the brain level tend to include effects on adult brains (Korshing, 1986), but behavioral functions involving NGF have not been taken into consideration.

Presence of GFs in Adult Vertebrates

It is well known that several vertebrate species synthesize and/or store at adulthood large amounts of GFs in particular structures. As far as NGF is concerned, as an example (NGF is the best known GF among the ever increasing GF molecule "family") (Mercola and Stiles, 1988; Sporn and Roberts, 1988), it is present in the salivary/venom gland of snake species belonging to the *Viperidae*, *Crotalidae*, and *Elapidae* families (Cohen, 1959; Levi-Montalcini and Cohen, 1965; Hogue-Angeletti et al., 1976; Levi-Montalcini and Angeletti, 1968) in the prostate gland of the Guinea pig, and in seminal plasma of Guinea pigs and the bull (Harper et al, 1979; Harper and Thoenen, 1980; Thoenen et al, 1987). In particular, NGF, together with EGF is produced and stored in very high amounts within the submaxillary salivary glands in the mouse species.

Historically, these murine glandular structures have been extremely important, since they provide the richest and the most easily available

and accessible source of both NGF and EGF. A "gift of nature," easy to extract, and extremely useful in the past for their biochemical characterization and for studying their biological roles. Only in very recent years techniques of recombinant DNA may have represented a powerful alternative in producing several GFs, and providing, as an example, highly purified EGF for human therapeutical uses (Nakagawa et al., 1985; Ito et al., 1986; Brown et al., 1986). Despite the fact that most of the scientific investigations carried out during the past thirty years concerned developmental aspects of NGF biology, the actual molecules used in these studies were from adult structures (salivary glands) of unknown functioning. It is really amazing to note how little curiosity originated from this discrepancy between actual biological investigation and actual source of biological material.

Despite the ever increasing number of investigations carried out in the field of GF biology (it has been estimated that more than one EGF paper per day is published) (Carpenter and Zendegui, 1986), no sound hypothesis has been produced until very recent years to explain why rodent salivary glands contain NGF and EGF in very high amounts, ranging from 350 to 500 ng/mg of wet weight. In the meanwhile, the ontogeny of the storing processes was fully characterized: NGF presence is sexually dimorphic, since male mice have up to ten-fold more salivary GF than female individuals (Caramia et al., 1962; Levi-Montalcini and Angeletti, 1964; Levi-Montalcini and Angeletti, 1968). GF production and storage closely follow testosterone hormone ontogeny, reaching the plateau stage when full sexual maturity is achieved (Levi-Montalcini and Angeletti, 1964). Adult castration abolishes (or at least, markedly reduces) salivary levels (Levi-Montalcini and Angeletti, 1968; Aloe & Levi-Montalcini, 1980; Barka, 1980). Breeding experience also affects salivary NGF levels. Salivaries of male and female mice without sexual experience, in fact, contain less NGF than those of experienced breeders (Aloe, personal communication, 1985).

Some of the hypothetical points of view expressed in the last decades stated that such a storage phenomenon in mice could be explained in terms of an epiphenomenal accumulation of biomaterial without any actual role in adult physiological regulation. In fact, glandular tissues evolve through rapid multiplication of unitary substructures; and such a multiplication could explain the high GF levels as accumulation of molecules of no biological value, in terms of "relics" of previous developmental stages, where they exerted an actual physiological role, and then "dragged" in the evolutionary process of multiplication (Barka, 1980; Thoenen & Barde, 1980; Purves & Lichtman, 1984). The bigger the glandular structure, the more GF molecules will be contained in it.

Other explanations have also been proposed, but none of them was consistent with a proposition of a physiological role for adult GF. All

hypotheses turned around the idea of the *lusus naturae*/freak molecule accumulation leitmotif.

A Biological Role for Mouse Salivary NGF

In 1983 Rita Levi-Montalcini and we started a new research program, aimed at assessing whether or not a series of behavioral syndromes could cause NGF release from male mouse salivary glands into the bloodstream. This idea arose out of several observations in the literature, claiming (Caramia et al., 1962; Aloe et al, 1985; Hendry & Iversen, 1973; Wallace & Partlow, 1976;), or negating (Ogata, 1955; Murphy et al., 1977b; Burton et al., 1978; Murphy et al., 1980) NGF presence in the bloodstream of mice. It appeared to us that such a series of conflicting results could be produced by some uncontrolled (behavioral) variable. Moreover, Bing and co-workers (Bing & Poulsen, 1979; Bing et al., 1980) found that renin, another protein molecule exerting *in vivo* strong regulatory properties, (which is also stored in male mouse salivary glands), was released into the bloodstream following intermale aggressive behavior caused by a prolonged period of individual housing.

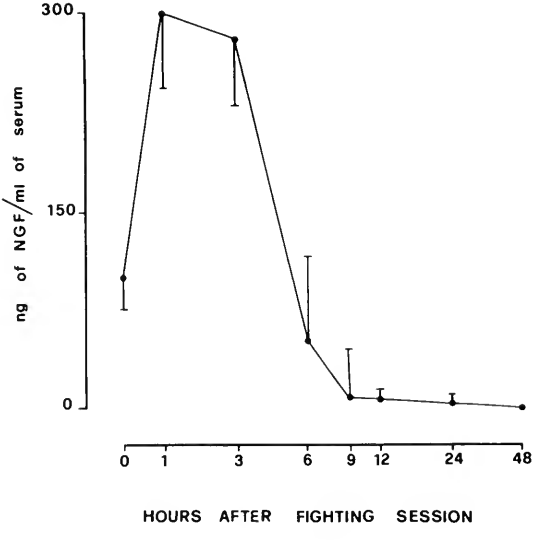

FIGURE 1

Time course of NGF release in the bloodstream of fighting mice. Fighting mice were paired for 20 min with an 8-week isolated adult male mouse, resulting in 32 to 38 separate fighting episodes. All mice used were of the Swiss-derived CD-1 outbred strain. Each point represents the mean ± SEM of six mice. Different animals were used for each time point. For more details see Aloe et al. 1986.

We found that NGF is released into the circulation following a minimum of 12-20 fighting episodes occurring within minutes; that NGF release is rapid (we found circulating NGF after about fifteen minutes from the fighting episodes), reaching a peak blood value about two-three hours later, and returning to basal levels in about 48 hours (Fig. 1 and 2). The peak value was about 300 μl/ml of serum, demonstrating that a massive NGF release actually occurred (Aloe et al., 1986).

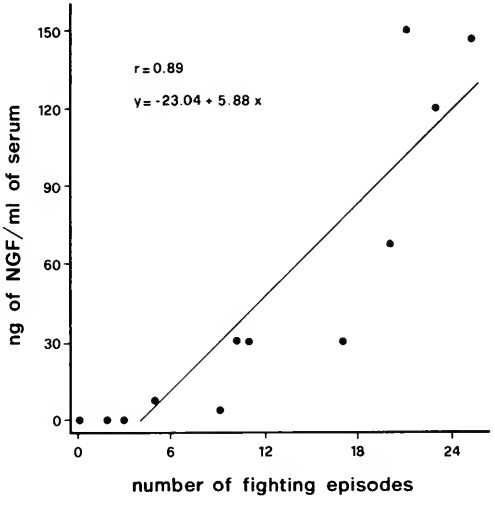

FIGURE 2

NGF levels in blood serum of fighting mice as a function of aggressive behavior scored during a 6 min session. For more details see Aloe et al., 1986.

Sialoadenalectomy (i.e. complete removal of salivary glands) results in undetectable NGF levels after fighting, although it cannot be ruled out that, using more sensitive techniques than those now available, small differences in NGF baseline quantities could be determined. There are many examples showing that endogenous NGF is secreted from source(s) other than the salivary glands (Murphy et al., 1977a; Thoenen & Barde, 1980; Taniuchi et al., 1988). It has been shown in fact that NGF is synthesized and released continuously in minute quantities by sympathetic and sensory neuronal target cells, and following nerve injury or axotomy (Goedert et al., 1986; Thoenen et al., 1987). However, whether or not these different sources of NGF are directly or indirectly activated by fighting behavior has to be established. Following fighting, immunohistochemical and electron microscopy examination (Fig. 3 and 4) of the glandular tissue confirmed a marked depletion of the ductal granules containing NGF, thus indicating that the great increase of blood NGF level was mainly due to secretion from salivary sources (Aloe et al., 1986).

From a behavioral point of view we found that NGF is released only by psychosocial stressors (Weiss, 1968; Axelrod, 1983; Axelrod & Reisine, 1984), i.e., following stress conditions involving behavioral interactions among conspecifics. In fact, we were unable to detect any difference in circulating NGF levels using stress conditions such as "cold water swim," escapable or inescapable footshock, forced biting, or forced restraint (Aloe et al., 1986). For ethical reasons we never tested shock-induced fighting.

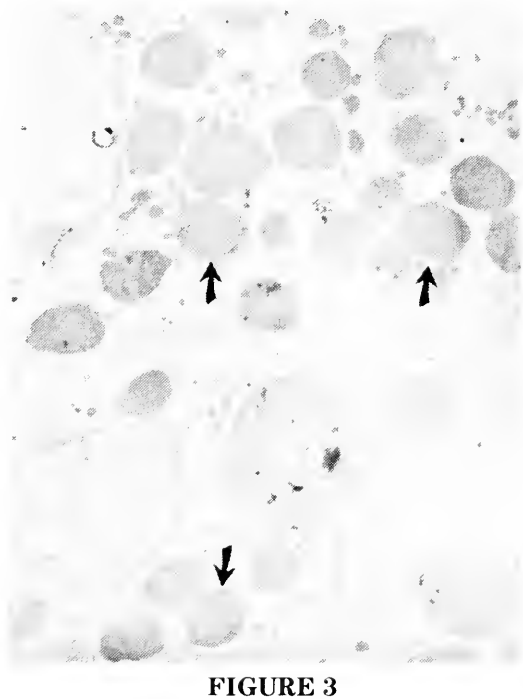

FIGURE 3

Electron micrograph of granule tubule cells from an adult male mouse salivary gland (control). NGF is stored within the electron-dense granules shown by arrows (x 6000). For more details see Aloe et al., 1986.

In contrast, both isolation-induced intermale fighting (and, at lower blood levels, interfemale aggressive behavior) caused a rise in NGF blood levels, as did also precopulation sexual arousal (Alleva & Aloe, 1988). It is rather well known that profound differences exist, in the physiological mechanisms involved in psychosocial stress syndromes and non-psychosocial syndromes (Coe & Levine in press; Henry et al., 1971; Axelrod & Reisine, 1984; Levine & Coe, 1985). Moreover, the specificity of the NGF release can be easily related to the way the animal copes with

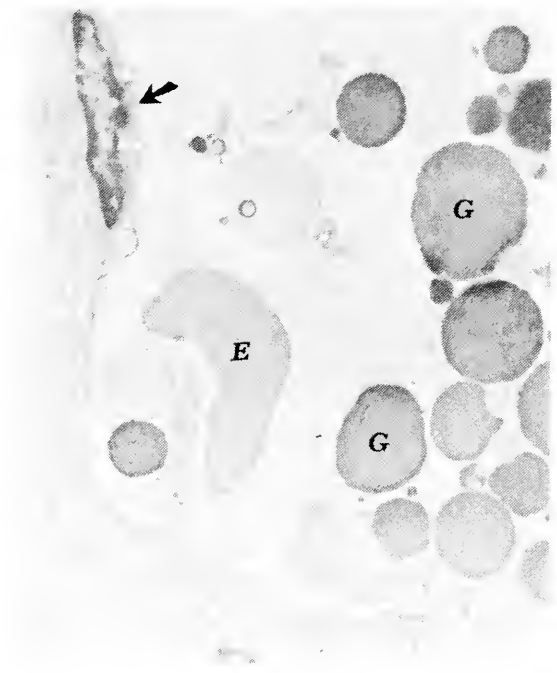

FIGURE 4

Electron micrograph showing a portion of blood vessel within the submaxillary salivary gland of a fighting male mouse. Electron-dense granules similar to those in the granular tubule cells are seen inside the blood vessel, indicating that these granules are released into the circulation. Arrow points to an endothelial cell lining a thin-walled vein, filled with erythrocytes (E) and electron-dense (salivary) granules (G). (x 7000).

the "stressing" situation, in terms of active attempts to put the situation under control, rather than to cope with it through a passive habituation to an unavoidable set of negative stimuli (Levine et. al., 1979; Levine, 1983; Levine & Coe, 1985). In other words, we can expect the animal will be able to display a particular set of social responses toward conspecifics, while interactions with the human experimenter or with inanimate objects will elicit a totally different cascade of physiological events. NGF release, which is accompanied by a concomitant release of salivary EGF (Bing & Poulsen, 1979, Bing et al., 1980; Aloe et al., 1986; Lakshamanan, 1986a), appears then to be causally linked to the first (social) category of "stressing" events. NGF and EGF now seem to be rather specific mediators in rodents (and useful markers) of a series of behavioral/physiological events triggered by a social confrontation inducing behavioral arousal (Fig. 5 and 7).

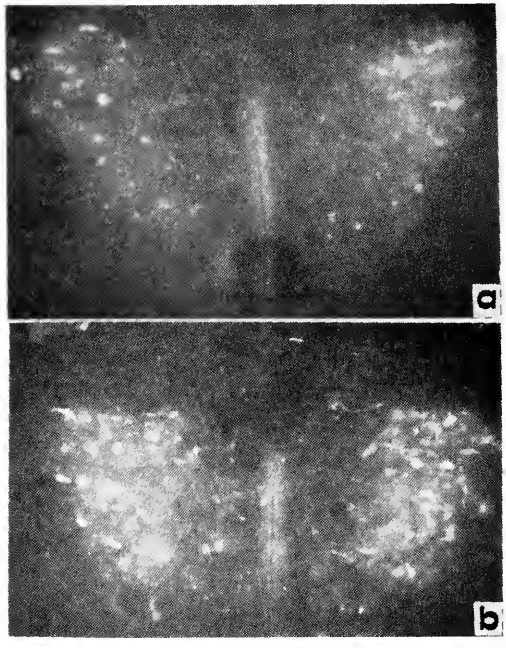

FIGURE 5

Immunofluorescence-stained sections of the anterior hypothalamic area of a control (a) and a fighting (b) adult male mouse showing more numerous NGF positive cells in (b) than in (a). (x 60). For more details see Aloe and Alleva, 1987.

A First Biological Target of Mouse NGF Release: The Adrenals

Several *in vivo* studies and miriads of *in vitro* investigations carried out in the past, at first by the pioneering work of Rita Levi-Montalcini and co-workers, and then by dozens of laboratories in the last three decades, have shown that NGF acts as a trophic and differentiative agent for the peripheral nervous system (Levi-Montalcini & Angeletti, 1968; Levi-Montalcini, 1987; Thoenen et al., 1987), and for the chromaffine tissue contained in the adrenal gland (Zaimis, 1971; Aloe & Levi-Montalcini, 1979). At the beginning it was quite obvious to look at these tissues as putative targets of a massive release of salivary NGF into the blood-stream following fighting.

In fact, it is known from the literature that male mouse adrenals change rather markedly (and rather quickly) following fighting behavior, while female adrenal morphology seems to be much more stable (Welch & Welch, 1969; Brain 1971; 1972; Hucklebridge, et al. 1981). As we reported above, male mice store an enormous amount of NGF in their salivary glands. It appeared therefore worthwhile to hypothesize that salivary NGF controls adrenal morphology (as well as adrenal functional

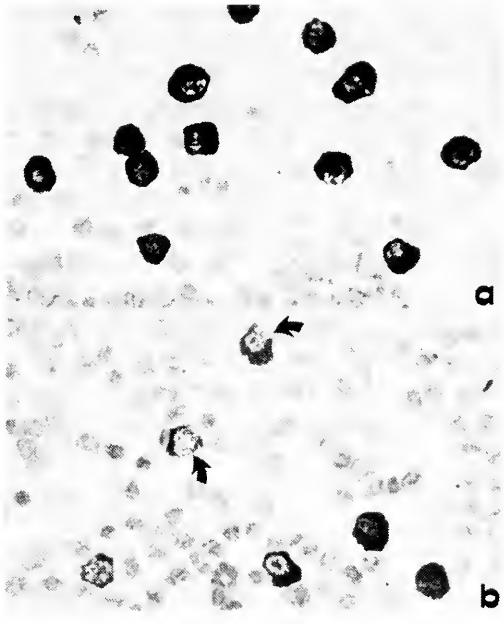

FIGURE 6

Peritoneal mast cells of isolated (a) and fighting (b) adult male mice showing plasma membrane disruption and cell degranualtion (arrows) following aggressive behavior. (x 160). A concomitant histamine release in the peritoneal fluid was found. For more details see Aloe, 1987.

status) through blood NGF levels, which in turn are regulated by the social/aggressive behavior (see the right part of Fig. 7). Moreover, mouse strains characterized by high levels of aggressive behavior have higher NGF levels in the bloodstream, as reported by Stephani et al., 1987.

We showed that exogenous NGF administrations (given intraperitoneally for ten consecutive days) markedly increase adrenal size (Aloe et al., 1986). It is interesting to note that both the medullary and the cortical layers increase their size following NGF exposure. These findings open the field to subsequent analyses of the putative mechanisms regulating relative balance between medullary and cortical zones of the adrenal gland, and involving interactions with NGF molecules.

It is almost trivial to underline the profound endocrine and behavioral changes produced by such a dramatic enlargement of the adrenals. We could expect both short-term changes in behavioral reactivity at the individual level and long-term changes in mouse population structure (Christian, 1965; Brain, 1971; Bronson, 1987). The latter could be caused by endocrine changes evoked by NGF release, affecting both peripheral nervous system reactivity, and adrenal hormonal levels. These changes are finely tuned by the frequency and

intensity of NGF release, i.e., by the fighting behavior displayed by each single animal within the population (Bronson, 1987; Drickamer, 1987). Moreover (see later) the concomitant EGF release affects male breeding capability directly (Tsutsumi et al., 1986). In other words, GF role in adult mice can be easily explained using an *in vivo* approach, and having in mind sound biological perspectives.

The Hypothalamic NGF Increase

We have recently reported that isolation-induced aggressive behavior causes a marked increase in hypothalamic NGF levels (Aloe & Alleva, 1987). This finding has been confirmed by several independent lines of evidence. NGF was first detected using immunohistochemical localization (Fig. 5), then confirmed by the classical NGF bioassay (halo effect in sympathetic chick ganglia) and radioimmunoassay. We are now carrying out *in situ* hybridization studies in order to measure normal $mRNA^{NGF}$ levels and/or fighting-induced changes of $mRNA^{NGS}$ levels in the hypothalamus.

The tentative explanation for such a difference in brain NGF could be, at first, that NGF interacts with other polypeptides, peptides, and/or hormones which are present in the hypothalamic area, affecting some feedback mechanism, and in turn changing the endocrine status of the organism (Swanson & Sawchenko, 1983; Albert, 1987). A series of interactive effects between NGF and thyroid hormones, ACTH, and peptides, have been reported in the past (Otten et al., 1979; Aloe & Levi-Montalcini, 1980; Otten, 1984; Wion et al., 1985). Recent *in vivo* findings have shown that NGF and thyroid hormones exert a synergistic effect on cholinergic cells of the CNS, suggesting an interaction between NGF and thyroid hormones in the regulation of choline acetyltransferase activity (Hayashi & Patel, 1987).

Such a brain change could in turn cooperate with the concomitant release in the periphery. In fact, we do not expect NGF to cross the blood/brain barrier, and we still do not know if any bioactive NGF fragment could actually enter the brain. However, it has been shown that monoclonal antibodies to the NGF receptor can pass from the cerebrospinal fluid to the region of the brain which contains NGF receptors (Schweitzer, 1987), suggesting that the NGF molecule could enter the brain in a similar way.

Another biological explanation, much more speculative than the former one (but not excluding it), is that the sudden presence of relatively high NGF levels within the brain could serve some still unknown process leading to a renewal of the brain plasticity at the adult stage. We know in fact that under particularly arousing behavioral contexts a "renewed plasticity" of the brain/behavioral condition has been reported

(Black, 1986; Black et al., 1987). It appears that stress can cause imprinting-like phenomena in adults, in which some environmental stimuli suddenly became relevant, and produce long-lasting behavioral alterations (Bateson, 1981; Bateson 1982; Albonetti & D'Udine, 1986). Furthermore, physiological significance of endogenous NGF in regulating the mechanisms of neuronal plasticity in the CNS of adult rodents has been recently suggested. It has been hypothesized that normally ongoing release of endogenous NGF evokes collateral sprouting from the terminal field in the adult nervous system, and that NGF could be involved in adult brain plasticity (Diamond et al., 1987).

Hypothalamic NGF could also represent a functional link between the "emotional" status caused by a psychosocial stressor and the biological needs of the organism (and of its brain) to "remember" the events leading to an appropriate (or inappropriate) coping with the stressor itself. NGF could shift some still unknown brain zones backwards to an "immature-like" stage. The only evidence we have at the present time are that (1) NGF-sensitive brain zones include cholinergic areas (cortex, hippocampus, nucleus basalis magnocellularis, etc.) (Gnahn et al. 1983; Shelton & Reichardt, 1986; Thoenen et al., 1987; Whittemore et al., 1987), and (2) these zones are classically considered the substrates of associative and retention processes. Experimental evidence published very recently have shown that several other structures within the CNS (both cholinergic and non-cholinergic) express NGF receptors (Korsching et al., 1985; Goedert et al., 1986; Shelton & Reichardt, 1986; Raivich & Kreutzberg, 1987; Pioro & Cuello, 1988), suggesting a rather complex role of the NGF molecule in regulating adult rodent CNS.

Endogenous NGF Release Induces Histamine Release from Mast Cells

The first evidence that NGF molecules interact with elements of the immune system was reported by Aloe & Levi-Montalcini (1977), showing that systemic treatment of newborn rats with NGF caused widespread accumulation of mast cells in subepiderma layers and other tissues. Subsequent *in vitro* and *in vivo* studies showed that other cell lines of the immune system of young and adult rodents, such as mast cells (Bruni et al., 1982; Sugiyama et al., 1985), lymphocytes (Abramchik et al., 1988), and platelets (Gudat et al., 1981) also respond to the action of NGF. Further evidence confirmed that NGF stimulates histamine release from isolated adult mast cells and induces plasma extravasation in rat skin. It has also been shown that NGF causes phenotypic conversion of spleen cells into mast cells (Böhm et al., 1986) and potentiates the blastogenic response (an *in vivo* model of immune system response) (Mazurek et al., 1986).

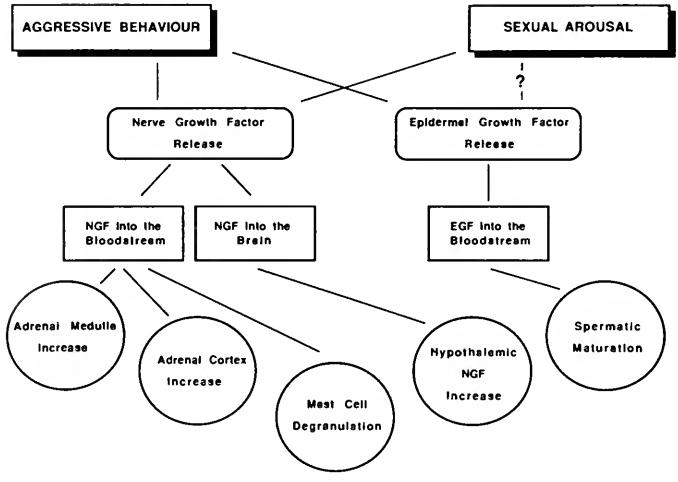

FIGURE 7

Diagram showing the behavioral states and the resulting physiological changes in which NGF and EGF are involved. No information is so far available on EGF release following (pre-copula) sexual arousal. See text for more details.

We have recently studied the structural, ultrastructural, and pharmacological response of peritoneal mast cells after massive release of endogenous NGF from mouse salivary glands. We observed that following isolation-induced aggressive behavior, male mouse mast cells of the peritoneum are highly degranulated (Fig. 7), and as a result of such a degranulation process, a concomitantly higher histamine level in their peritoneal fluid was found (to be published). Exposure to anti-NGF antibodies blocks mast-cell degranulation, indicating that salivary NGF is specifically involved in mast-cell activation. Furthermore, sialoadelanectomy in adult male mice or chronic exposure to NGF antibodies causes size reduction in peritoneal cells, particularly mast cells.

Such a series of results suggest a functional role of NGF, occurring as a result of psychosocial stress, i.e., the potentiation of the immune defenses of the organism, when it faces a potentially damaging life event. However, it has to be stressed that specific stress syndromes cause rather specific changes in specific portions of the immune systems (Stein et al., 1985; Coe & Levine, in press). In particular, as far as intermale fighting in mice is concerned, evidence that fighting results in immunological activation has been reported (Hadry et al., 1987). The NGF release, and the resulting mast-cell degranulation, appears to be a rather selective and limited effect of immunological defence activation.

NGF and EGF Release as Factors Controlling Mouse Populations

Other authors have reported that EGF is similarly co-released with NGF into the bloodstream following isolation-induced intermale fighting (Lakshamanan, 1986b). EGF is also dimorphically contained in much higher amounts in the salivary glands of male mice, while females show low storage levels (Cohen, 1962). It is not yet clear whether or not salivary EGF is stored in the same granules which contain NGF, or in different granules, but their release after aggressive encounters follows approximately the same pattern. Such a co-release poses questions as to whether or not the same cell targets are involved, since a number of different nerve cell types exhibit both NGF and EGF receptors (Levi-Montalcini, 1987; Gomez-Pinilla, 1988; Pioro & Cuello, 1988; Werner et al., 1988). Another point, supposing that both GFs are stored in the same granule, is whether their releases are functionally linked, or viceversa elicited by independent mechanisms. Very recent observations indicate that both EGF and NGF, and $mRNA^{NGF}$ are present in rat testes and seminal fluid (Tsutsumi et al. 1986; Olson et al., 1987), raising some interesting questions about NGF involvement in sperm maturation and motility.

Tsutsumi et al. (1986) recently provided a sound biological explanation for EGF presence in mouse salivary glands. These authors showed that EGF is continuously released into circulation, where it can be detected at the 5 ng/ml level. Sialoadenalectomy abolishes circulating EGF levels, since no detectable EGF was found in serum following salivary gland removal. More interestingly, the number of mature sperm in the epididymis decreased by as much as 55%. At the same time, the number of spermatid in the testes decreased by 40 to 50%, while the number of spermatocytes increased by about 20%. EGF administration to sialoadenalectomized mice restored to normal values both the sperm content of the epididymis and the number of spermatids in the testes. This study demonstrated that, in adult mice, EGF may play a key role in male reproductive function by stimulating the meiotic phase of spermatogenesis.

A subsequent study showed that EGF is also released in male mice following aggressive interactions caused by social isolation (Lakshamanan, 1986b). It is possible to speculate that EGF, together with NGF, exert a "tonic" influence on male reproductive/endocrine status, which in turn depends on the frequency of (and/or amount of) aggressive encounters. Such a functional role could be an essential feature in regulating mouse population levels under natural conditions. Aggressive behavior caused by overcrowding, limited resources, etc. could in fact shape the composition of murine population through hormonal/repro-

ductive/behavioral alterations (Christian et al. 1965; Brain, 1971; Bronson, 1987; Drickamer, 1987) modulated by NGF/EGF release.

REFERENCES

Abramchik, G. V., Yermakova, S. S., Kaliunov, V. N., Tanina, R. M. & Tumilovich, M. K. (1988). The immunomodulatory effect of nerve growth factor. *Journal of Neuroscience Research, 19*, 349-356.

Albonetti, M. E. & D'Udine, B. (1986). Social experience occurring during adult life: its effects on socio-sexual olfactory preferences in inbred mice, *Mus musculus*. *Animal Behaviour, 34*, 1844-1847.

Albert, D. J., Dyson, E. M., Walsh, M. L. & Gorzalka, B. B. (1987). Intermale social aggression in rats: suppression by medical hypothalamic lesions independently of enhanced defensiveness or decreased testicular testosterone. *Physiology and Behaviour, 39*, 693-698.

Alleva, E. & Aloe, L. (1988). Aggressive behavior, sex arousal, and NGF release in adult mice. *Animal Behaviour*, Abstract of oral presentation, ASAB-SCAB Meeting, Edinbourgh (in press).

Aloe, L. & Alleva, E. (1987). Nerve growth factor increases in the bloodstream and hypothalamus of aggressive mice. Abstract. 5th Capo Boi Conference on Neuroscience, Villasimius, Sardenia.

Aloe, L., Alleva, E., Bohm, A. & Levi-Montalcini, R. (1986). Aggressive behavior induces release of nerve growth factor from mouse salivary gland into blood stream. *Proceedings of the National Academy of Sciences*, (USA) *83*, 6184-6187.

Aloe, L., Cozzari, C. & Levi-Montalcini, R. (1985). Cyclocytidine-induced release of nerve growth factor from mouse submandibular glands enhances regeneration of sympathetic fibers in adult mice. *Brain Research, 332*, 259-265.

Aloe, L. & Levi-Montalcini, R. (1977). Mast cells increase in tissues of neonatal rats injected with the nerve growth factor. *Brain Research, 133*, 358-366.

Aloe, L. & Levi-Montalcini, R. (1979). Nerve growth factor in vivo induced transformation of immature chromaffin cells in sympathetic neurons: effect of antiserum to the nerve growth factor. *Proceedings of the National Academy of Sciences* (USA), *76*, 1246-1250.

Aloe, L. & Levi-Montalcini, R. (1980). Comparative studies on testosterone and L-thyroxine effects on the synthesis of nerve growth factor in mouse submaxillary salivary glands. *Experimental Cell Research, 125*, 15-22.

Axelrod, J. (1983). The relationship between the stress hormones, catecholamines, ACTH and glucocorticoids. In E. Usdin, R. Kvetnansky & J. Axelrod (Eds.), *Stress*, pp. 120-131, New York: Gordon and Breach Science Publishers.

Axelrod, J. & Reisine, T. D. (1984). Stress hormones: Their interaction and regulation. *Science, 224*, 452-459.

Barka, T. (1980). Biologically active polypeptides in submanibular glands. *Journal of Histochemistry and Cytochemistry, 28*, 836-859.

Bateson, P. P. G. (1981). Ontogeny of behavior. *British Medical Bulletins, 37*, 159-164.

Bateson, P. P. G. (1982). Sensitive periods and the possibility for renewed plasticity later. Abstract. European Brain and Behaviour Society Meeting, Groningen.

Bing, J. & Poulsen, K. (1979). In mice aggressive behaviour provokes vast increase in plasma renin concentration, causing only slight, if any, increase in blood pressure. *Acta Physiologica Scandinavica, 105*, 64-72.

Bing, J., Poulsen, K., Hackenthal, E., Rix, E. & Taugner, R. (1980). Renin in the submaxillary gland: a review. *Journal of Histochemistry and Cytochemistry, 28*, 874-880.

Black, I. B. (1986). Trophic molecules and evolution of the nervous system. *Proceedings of the National Academy of Sciences*, (USA) 83, 8249-8252.

Black, I. B., Adler, J. E., Dreyfus, C. F., Friedman, W. F., LaGamma, E. F. & Roach, A. H. (1987). Biochemistry of information storage in the nervous system, *Science 236*, 1263-1268.

Bohm, A. Aloe, L. & Levi-Montalcini, R. (1986). Nerve growth factor enhances precocious differentiation and numerical increase in mast cells in cultures of rat splenocytes. *Rendiconti Accademia Nazionale dei Lincei, 80,* 1-6.

Brain, P. F. (1971). The physiology of population limitation in rodents—A review. *Communications in Behavioral Biology, 6,* 115-123.

Brain, P. F. (1972). Endocrine and behavioral differences between dominant and subordinate male house mice housed in pairs. *Psychonomic Science, 28,* 260-262.

Bronson, F. H. (1987). Environmental regulation of reproduction in rodents. In D. Crews (Ed.), *Psychobiology of reproductive behavior,* pp. 204-230, Englewood Cliffs, N. J.: Prentice Hall.

Brown, G. L., Curtsinger, L. III, Brightwell, J. R., Ackerman, D. M., Tobin, G. R., Polk, H. C. Jr., George-Nascimento, C., Valenzuela, P. & Schultz, G. S. (1986). Enhancement of epidermal regeneration by biosynthetic epidermal growth factor. *Journal of Experimental Medicine, 163,* 1319-1324.

Bruni, A., Bigon, E., Boarato, E., Mietto, L., Leon, A. & Toffano, G. (1982). Interaction between nerve growth factor and lysophosphatidyl- serine on rat peritoneal mast cells. *FEBS Letters, 138,* 190-193.

Burton, L. E., Wilson, W. H. & Shooter, E. M. (1978). Nerve growth factor in mouse saliva. *Journal of Biological Chemistry, 253,* 7807-7812.

Calissano, P., Cattaneo, A., Aloe, L. & Levi-Montalcini, R. (1984). The nerve growth factor. In C. H. Li (Ed.), *Hormonal proteins and peptides,* New York: Academic Press.

Caramia, F., Angeletti, P. U. & Levi-Montalcini, R. (1962). Experimental analysis of the mouse submaxillary salivary gland in relationship to its nerve-growth factor content. *Endocrinology, 70,* 915-922.

Carpenter, G. & Zendegui, J. G. (1986). Epidermal growth factor, its receptor, and related proteins. *Experimental Cell Research, 164,* 1-10.

Christian, J. J., Lloyd, J. A. & Davis, D. E. (1965). The role of endocrines in the self-regulation of mammalian populations. In G. Pincus (Ed.), *Recent progress in hormone research,* Vol. 21, pp. 501-578, New York: Academic Press.

Coe, C. L. and Levine, S. Behavioral immunology: An old idea that's time has come. In P. Barchas (Ed.), *Sociophysiology of social relationships* (in press).

Cohen, S. (1959). Purification and metabolic effects of a nerve growth-promoting protein from snake venom. *Journal of Biological Chemistry, 234,* 1129-1137.

Cohen, S. (1962). Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. *Journal of Biological Chemistry, 237,* 1555-1562.

Diamond, J., Coughlin, M., Macintyre, L., Holmes, M. & Visheau, B. (1987). Evidence that endogenous β nerve growth factor is responsible for the collateral sprouting, but not the regeneration, of nociceptive axons in adult rats. *Proceedings of the National Academy of Sciences* (USA), *84,* 6596-6600.

Drickamer, L. C. (1987) Behavior as a factor in the population dynamics of rodents: New concepts and approaches (Introduction to the special issue) *American Zoologist, 821,* 821-25.

Fallon, J. H., Serrogy, K. B., Longhlin, S. E., Morrison, R. S., Bradshaw, R. A, & Cunningham, D. D. (1984). Epidermal growth factor immunoreactive material in the central nervous system: location and development. *Science, 224,* 1107.

Gnahn, H., Hefti, F., Heumann, R., Schwab, M. E. & Thoenen, H. (1983). NGF-mediated increase of choline acetyltransferase (ChAT) in the neonatal rat forebrain, evidence for a physiological role of βNGF in the brain? *Developmental Brain Research, 3,* 229-238.

Goedert, M., Fine, A., Hunt, S. P. & Ullrich, A. (1986). Nerve growth factor mRNA in peripheral and central rat tissues and in the human central nervous system: Lesion effects in the rat brain and levels in Alzheimer's disease. *Molecular Brain Reserch, 1,* 85-92.

Gomez-Pinilla, F., Knauer, D. J. & Nieto-Sampedro, M. (1988). Epidermal growth factor receptor immunoreactivity in rat brain: Development and cellular localization. *Brain Research, 438,* 385-390.

Gudat, F., Laubscher, A., Otten, U. & Pletscher, A. (1981). Shape changes induced by biologically active peptides and nerve growth factor in blood platelets of rabbits. *British Journal of Pharmacology, 74,* 533-538.

Hadry, J., J. Quay, R. Ader, & Linvat, S. (1987) Aggressive behavior and dominance modify immune response in mice. *Society of Neuroscience Abstract, 13,* Abstract No. 438.9.

Harper, G. P., Barde, Y. A., Burnstock, G., Carstairs, J. R., Dennison, M. E., Suda, K. & Vernon, C. A. (1979). Guinea pig prostate is a rich source of nerve growth factor. *Nature, 279,* 160-162.

Harper, G. P. & Thoenen, H. (1980). The distribution of nerve growth factor in the male sex organs of mammals. *Journal of Neurochemistry, 77,* 391-401.

Hayashi, M. & Patel, A. J. (1987). An interaction between thyroid hormone and nerve growth factor in the regulation of choline acetyltransferase activity in neuronal cultures, derived from the septal-diagonal band region of the embryonic rat brain. *Developmental Brain Research, 36,* 109-120.

Hendry, I. A. & Iversen, L. L. (1973). Reduction in the concentration of nerve growth factor in mice after sialectomy and castration. *Nature, 243,* 500-504.

Henry, J. P., Stephens, P. M., Axelrod, J. & Mueller, R. A. (1971). Effect of psychosocial stimulation of the enzymes involved in the biosynthesis and metabolism of noradrenaline and adrenaline. *Psycosomatic Medicine, 33,* 227-237.

Hogue-Angeletti, R. A., Frazier, W. A., Jacobs, J. W., Niall, H. D. & Bradshaw, R. A. (1976). Purification, characterization, and partial amino acid sequence of nerve growth factor from cobra venom. *Biochemistry, 15,* 26-34.

Hucklebridge, F. H., Gamal-el-Din, L. & Brain, P. F. (1981). Social status and the adrenal medulla in the house mouse. *Behavioural and Neural Biology, 33,* 345-363.

Ito, A., Katoh, T., Gomi, H., Kishimoto, F., Agui, H., Ogino, S., Yoda, K., Yamasaki, M. & Tamura, G. (1986). Expression and secretion of human epidermal growth factor in Escherichia coli. *Agricultural Biology and Chemistry, 50,* 1381-1388.

Korsching, S. (1986). Nerve growth factor in the central nervous system. *Trends in Neuroscience, 9,* 570-573.

Korsching, S., Auburger, G., Heumann, R., Scott, J. & Thoenen, H. (1985). Levels of nerve growth factor and its mRNA in the central nervous system of the rat correlate with cholinergic innervation. *European Molecular Biology Organization Journal, 4,* 1389-1393.

Lakshamanan, J. (1986a). Nerve growth factor levels in mouse serum: Variations due to stress. *Neurochemistry Research 12,* 393-397.

Lakshamanan, J. (1986b). Aggressive behavior in adult male mice elevates serum nerve growth factor levels. *American Journal of Physiology, 250,* 386-392.

Levi-Montalcini, R. (1987). The nerve growth factor: Thirty-five years later. *Science, 237,* 1154-1162.

Levi-Montalcini, R. & Angeletti, P. U. (1964). Hormonal control of the NGF content in the submaxillary salivary glands of mouse. In L. M. Sreebny & J. Meyer (Eds.). *Salivary glands and their secretions,* pp. 129-144. Oxford: Pergamon.

Levi-Montalcini, R. & Angeletti, P. U. (1968). The nerve growth factor. *Physiological Review, 48,* 534-569.

Levi-Montalcini, R. & Cohen, S. (1965). In vitro and in vivo effects of a nerve growth factor-stimulating agent isolated from snake venom. *Proceedings of the National Academy of Sciences* (USA), *42,* 695-699.

Levine, S. (1984). A psychobiological approach to the ontogeny of coping. In N. Garmezy & M. Rutter (Eds.), *Stress, coping, and development in children,* pp. 107-131. New York: McGraw-Hill.

Levine, S. & Coe, C. L. (1985). The use and abuse of cortisol as a measure of stress. In T. M. Field, P. M. McCabe & Schneiederman (Eds.), *Stress and coping,* pp. 149-159. New York: Hillsdale, NJ: Earlbaum.

Levine, S., Weinberg, J. & Brett, L. (1979). Inhibition of pituitary-adrenal activity as a consequence of consummatory behavior. *Psychoneuroendocrinology, 4,* 275-286.

Lillien, L. E. & Claude, P. (1985). Nerve growth factor in a mitogen for cultured chromaffin cells. *Nature, 317,* 632-634.

Mazurek, N., Weskamp, G., Erne, P. & Otten, U. (1986). Nerve growth factor induces mast cell degranulation without changing intracellular calcium levels. *FEBS Letters, 198,* 315-320.

Mercola, M. & Stiles, C. D. (1988). Growth factor superfamilies and mammalian embryogenesis. *Development, 102,* 451-460.

Murphy, R. A., Oger, J., Saide, J. D., Blanchard, M. H., Arnason, B. G. W., Hogan, C., Pantazis, N. J. & Young, M. (1977a). Secretion of nerve growth factor by central nervous system glioma cells in culture. *Journal of Cellular Biology, 72,* 769-773.

Murphy, R. A., Saide, J. D., Blanchard, M. H. & Young, M. (1977b). Nerve growth factor in mouse serum and saliva: role of the submandibular gland. *Proceedings of the National Academy of Sciences,* (USA), *74,* 2330-2333.

Murphy, R. A., Watson, A. Y., Metz, J. & Forssmann, W. G. (1980). The mouse submandibular gland. *Journal of Histochemistry and Cytochemistry, 28,* 890-902.

Nakagawa, S., Yoshida, S., Hirao, Y., Kasuga, S. & Fuwa, T. (1985). Biological effects of biosynthetic human EGF on the growth of mammalian cells in vitro. *Differentiation, 29,* 284-288.

Ogata, T. (1955). The internal secretion of salivary gland. *Japanese Endocrinology 12,* 247-262.

Olson, L., Ayer-LeLievre, C., Ebendal, T. & Seiger, A. (1987). Nerve growth factor-like immunoreactivities in rodent salivary glands and testis. *Cell and Tissue Research, 248,* 275-286.

Otten, U. (1984). Nerve growth factor and the peptidergic sensory neurons. *Trends in Pharmacological Sciences, July,* 307-310.

Otten, U., Baumann, J. B. & Girard, J. (1979). Stimulation of the pituitary-adrenocortical axis by nerve growth factor. *Nature, 282,* 413-414.

Pioro, E. P. & Cuello, C. A. (1988). Purkinje cells of adult rat cerebellum express nerve growth factor receptor immunoreactivity: light microscopic observations. *Brain Research, 455,* 182-186.

Purves, D. & Lichtman, J. W. (1984). *Principles of neural development.* Sunderland, MA: Sinauer Associates Inc. Publishers.

Raivich, G. & Kreutzberg, G. W. (1987). The localization and distribution of high affinity β-nerve growth factor binding sites in the central nervous system of the adult rat. A light microscopic autoradiographic study using (^{125}I)β-nerve growth factor. *Neuroscience, 20,* 23-36.

Schweitzer, J. B. (1987). Nerve growth factor receptor-mediated transport from cerebrospinal fluid to basal forebrain neurons. *Brain Research, 423,* 309-317.

Seiler, M. & Schwab, M. E. (1984). Specific retrograde transport of nerve growth factor from the neocortex to nucleus basalis in the rat. *Brain Research, 300,* 33-39.

Shelton, D. L. & Reichardt, L. F. (1986). Studies on the expression of the β nerve growth factor (NGF) gene in the central nervous system: Level and regional distribution of NGF mRNA suggest that NGF functions as a trophic factor for several distinct populations of neurons. *Proceedings of the National Academy of Sciences* (USA), *83,* 2714-2718.

Sporn, M. B. & Roberts, A. B. (1988). Peptide growth factors are multifunctional. *Nature, 332,* 217-219.

Stein, M., Keller, S. E. & Schleifer, S. J. (1985). Stress and immunomodulation: The role of depression and neuroendocrine function. *Journal of Immunology, 135,* 827-833.

Stephani, U., Sutter, A. & Zimmermann, A. (1987). Nerve growth factor (NGF) in serum: Evaluation of serum NGF levels with a sensitive bioassay employing embryonic sensory neurons. *Journal of Neuroscience Research, 17,* 25-35.

Sugiyama, K., Suzuki, Y. & Furuta, H. (1985). Histamine release induced by 7S nerve growth factor of mouse submandibular salivary glands. *Archives of Oral Biology, 30,* 93-95.

Swanson, L. W. & Sawchenko, P. E. (1983). Hypothalmamic integration: Organization of the paraventricular and supraoptic nuclei. *Annual Review of Neuroscience, 6,* 269-324.

Taniuchi, M., Clark, H. B., Schweitzer, J. B., & Johnson, E. M. Jr. (1988). Expression of nerve growth factor receptors by Schwann cells of axotomized peripheral nerves: Ultrastructural location, suppression by axonal contact, and binding properties. *Journal of Neuroscience, 8,* 664-681.

Thoenen, H., Bandtlow, C. & Heumann, R. (1987). The physiological function of nerve growth factor in the central nervous system: Comparison with the periphery. *Physiological and Biochemistry and Pharmacology, 109,* 146-171.

Thoenen, H. & Barde, Y. A. (1980). Physiology of nerve growth factor. *Physiological Review, 60,* 1284-1335.

Tsutsumi, O., Kurachi, H. & Oka, T. (1986). A physiological role of epidermal growth factor in male reproductive function. *Science, 233,* 975-978.

Wallace, L. J. & Partlow, L. M. (1976). A-Adrenergic regulation of secretion of mouse saliva rich in nerve growth factor. *Proceedings of the National Academy of Sciences* (USA), *73,* 4210-4214.

Weiss, J. M. (1968). Effects of coping responses of stress. *Journal of Comparative and Physiological Psychology, 65,* 251-260.

Welch, B. L. & Welch, A. S. (1969). Sustained effects of brief daily stress (fighting) upon brain and adrenal catecholamines and adrenal, spleen, and heart weights of mice. *Proceedings of the National Academy of Sciences* (USA), *64,* 100-109.

Werner, M. H., Nanney, L. B., Stoscheck, C. M. & King, L. E. (1988). Localization of immunoreactive epidermal growth factor receptors in human nervous system. *Journal of Histochemistry and Cytochemistry, 36,* 81-86.

Whittemore, S. R. & Seiger, A. (1987). The expression, localization and functional significance of β-nerve growth factor in the central nerve system. *Brain Research Review, 12,* 439-464.

Wion, D., Barrand, P., Dicou, E., Scott, J. & Brachet, P. (1985). Serum and thyroid hormones T3 and T4 regulate growth factor mRNA levels in mouse L cells. *FEBS Letters, 189,* 37-42.

Zaimis, E. (1971). Nerve growth factor: The target cells. In E. Zaimis & J. Kight (Eds.) *Nerve growth factor and its antiserum.* London: Athlone.

The International Journal of Comparative Psychology, Vol. 2, No. 4, Summer 1989

TACTILE CUES, BILATERALLY ASYMMETRICAL LEG MOVEMENTS AND BODY DISTORTION IN ISOPOD TURN ALTERNATION

Robert N. Hughes
University of Canterbury

ABSTRACT: Because woodlice *(Porcellio scaber)* and pillbugs *(Eluma purpurascens)* that traveled closer to the outer wall of alleys both before as well as after a 90° forced turn subsequently made sharper free turns in the opposite direction, it seemed possible that a quantitative relationship existed between tactile stimulation arising from wall contact and free-turn behavior. However, on emerging from straight runways, without any forced turns, pillbugs turned at sharper angles than woodlice but there was no relationship between the size of a turn and amount of wall contact apart from a very minor one for woodlice only. It was concluded that tactile stimuli played a negligible part in turn alternation of either species thereby supporting involvement of proprioceptive cues. By requiring woodlice to negotiate a forced turn lined with glass on the outer half of the floor, it became apparent that their alternation was determined by proprioceptive feedback from bilaterally asymmetrical leg movements rather than distortion of body segments.

Patterns of alternating turns at successive choice points and obstacles while free-moving have been described for a wide range of species (Hughes, 1989). In the absence of directional cues, such patterns provide a "correcting" influence (Barnwell, 1965; Dingle, 1964) which ensures relatively direct movement towards or away from significant environmental stimuli (Hughes, 1967, 1978). Sequential alternation should therefore facilitate foraging, exploratory and escape behavior as well as dispersal of the species (Hughes, 1978; Richman, Dember & Kim, 1987).

Since body turn alternation in most invertebrates depends mainly on response-generated proprioceptive rather than exteroceptive cues (Hughes, 1985), the phenomenon reflects an orientation reaction based on information about previous movement sequences. It has been proposed that the controlling mechanism in woodlice *(Porcellio scaber)* involves differential activity of legs on each side of the body during turning i.e., bilaterally asymmetrical leg movements (BALM, Hughes,

Address correspondence to Dr. R. N. Hughes, Department of Psychology, University of Canterbury, Christchurch 1, New Zealand.

1985). Alternation was seen to arise from the greater influence of legs on the side that had traveled the shorter distance or exerted less effort while negotiating a previous turn. Evidence for this view is found in turning to the same side as preceding unilateral forced walking (Beale & Webster, 1971), and traveling closer to the outer wall of an exit alley following sharper forced turns along with correlations between closeness to a wall and the size of a subsequent free turn (Hughes, 1985).

However, it has been recently shown that, while BALM influences appear to underly most woodlouse alternation, tactile cues can also be important for some individuals that come into close proximity with a vertical surface (Hughes, 1987). In spite of suggestions that, by itself, wall-following cannot account for the size of an alternating turn in either woodlice (Hughes, 1985) or centipedes (Schäfer, 1972), it is conceivable that exceptionally intense or prolonged tactile stimulation might modify the effects of a proprioceptive mechanism on alternation magnitude, particularly since slightly sharper turns may follow longer distances in contact with straight walls (Schäfer, 1976). In an attempt to clarify this issue, relationships between proximity to both pre- and post-forced turn outer walls and free turn angle size were examined in two isopod crustaceans, namely the woodlouse or sowbug *(P. scaber)* and a species of pillbug *(Eluma purpurascens)*. As more recent isopod studies have been confined to *P. scaber* (Hughes, 1985, 1987), pillbugs were included for comparative purposes since the tendency of a related species *(Armadillidium vulgare)* to alternate is well established, e.g., Iwata and Watanabe (1957), Kupfermann (1966). *P. scaber*, a member of the family Porcellionidae, was introduced to New Zealand over 150 years ago presumably by ship from the British Isles where it is indigenous. It is widespread and generally prefers a cosmopolitan habitat living under stones and wood particularly in home gardens (Hurley, 1950). *E. purpurascens* belongs to the family Armadillidiidae noted for its ability to roll up into a ball (conglobate) when threatened. It is also an introduced species which is very rare in the British Isles (Sutton, 1972), but it is common in Christchurch gardens even though its presence in New Zealand has not yet been formally recognised (Johns, 1989).

Rather than leg movement cues, Schäfer (1982) has suggested that proprioceptive feedback from displaced body segments determines the size of a subsequent alternating turn in isopods. This proposal does not appear to explain relationships between proximity to the outer wall of a post-forced turn exit alley (and forced-turn angle size) which can however be accounted for by BALM effects (Hughes, 1985). Nevertheless, a direct test of the two explanations was thought desirable even though either or neither might apply to other species with different leg and tagmata characteristics.

EXPERIMENT 1

When woodlice travel along the exit-alley of a runway following a forced turn, distance from the outer wall appears to reflect the pre-choice operation of BALM effects rather than determine free-turn behavior (Hughes, 1985). If the amount of tactile stimulation provided by this wall does not affect the size of a free turn, there should be no relationship between free-turn angle and proximity to the outer wall of the start alley preceding a forced turn. Experiment 1 therefore investigated the importance of nearness to pre- as well as post-forced turn outer walls in alternation behavior of woodlice and pillbugs. The contribution of the type of response made while negotiating the forced turn was also considered.

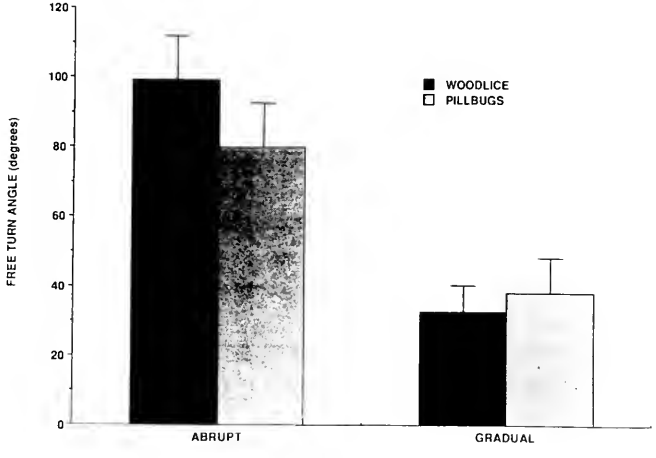

FIGURE 1

Mean + SE free-turn angle for abrupt and gradual turning woodlice and pillbugs following one 90° forced turn.

Method

Animals.—All woodlice and pillbugs used in this and later experiments were collected from beneath stones and decaying vegetation. They were kept in plastic receptacles containing damp soil, leaf litter and sliced carrot and potato. Only animals 4-6 mm in width were used. Fortyeight members of each species were observed in Experiment 1.

Apparatus.—For this experiment, the apparatus comprised a 10-mm-wide runway cut from 6-mm-thick clear Perspex with a 90° forced turn, a clear Perspex cover and an open-ended exit. The outer walls of the

start and exit alleys were 70 and 40 mm long respectively. The runway was positioned with the centre of a 200-mm-diameter circle (drawn on white paper) in the middle of the exit. To facilitate measurements of distances from the outer walls, there was a series of parallel lines drawn on the section of paper underlying the two alleys. At right angles to these, lines also appeared across the alleys at 10-mm intervals. A television camera (with a magnifying lens) and a VHS video-recorder were used to record behavior of the subjects while in the runway.

PROCEDURE

Each subject was gently lowered into the beginning of the start alley. The Perspex cover was replaced and the animal's progress from start to exit was video-recorded. On leaving the runway, the point where the subject crossed the circumference of the circle (r = 100 mm) was noted to enable subsequent determination of its free-turn angle of emergence. The video-record was later replayed and images frozen at distances of 30, 20, 10 and 0 mm from the forced turn to start and from the forced turn to exit. At each of these points, distances between the outer wall and the nearest part of each subject's thorax were measured. As the animal moved closer to this wall, greater numbers of those sense organs believed to be tactile receptors would have been stimulated. These organs comprise short bristles found on the dorsal and ventral surfaces, the legs and between the dorsal tergites, and in particular, long spines or "parking antennae" laterally positioned on each thoracic tergite (Jans & Ross, 1963). It was also noted whether the animal had collided with the exit alley outer wall before making its forced turn, thereby usually necessitating an "abrupt" right angle turn, or if it had begun turning into the exit alley before this wall had been encountered thus making a more "gradual" curved turn. Equal numbers of subjects were forced left and right.

RESULTS AND DISCUSSION

Numbers of abrupt and gradual turners were 11 and 37 respectively for woodlice and 19 and 29 for pillbugs. These proportions did not differ between the two species [χ^2 (1) = 2.38].

Free-turn angles for abrupt and gradual turners of each species are outlined in Figure 1. As shown by an ANOVA, abrupt turners turned at significantly sharper angles than gradual turners [F (1, 92) = 29.94, $p < .001$]. There was no significant species differences [F (1, 92) < 1] or Species X Turner interaction [F (1, 92) = 1.29] for this measure.

Fortythree (90%) woodlice and 39 (81%) pillbugs alternated [$p < .0006$ in both cases, binomial test], but the difference between these

proportions was not significant [$\chi^2(1) < 1$]. However, significantly more abrupt turners alternated (100%) than gradual turners [83%, $\chi^2(1) = 4.12$, $p < .05$].

Average distances from the outer wall for the four measurements taken both before (pre) and after the forced turn (post) were calculated for each subject. Results for abrupt and gradual turners of each species can be seen in Figure 2.

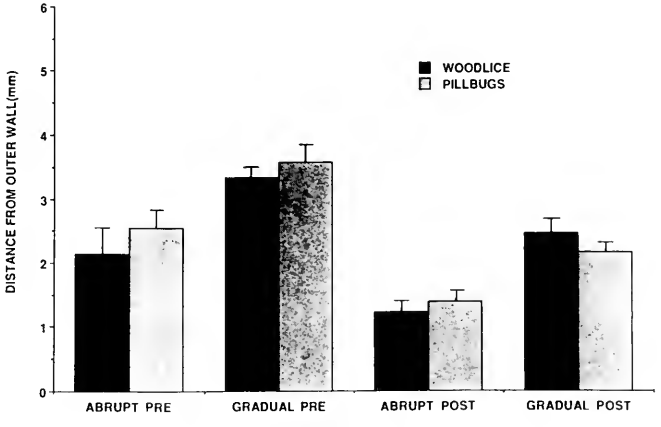

FIGURE 2

Mean + SE distance from the outer wall before (pre) and after (post) one 90° forced turn for abrupt and gradual turning woodlice and pillbugs.

While there was no significant species differences [$F(1,92) = 1.45$] abrupt turners traveled significantly closer to the two walls combined than gradual turners [$F(1, 92) = 25.06$, $p < .001$]. This difference characterised both the start [$t(92) = 7.79$, $p < .001$] and exit alley measurements [$t(92) = 5.79$, $p < .001$]. All subjects traveled closer to the outer wall of the exit alley than to that of the start alley [$F(1, 92) = 64.35$, $p < .001$]. No interactions were significant.

Correlations between free-turn angle and average distance from each outer wall were determined separately for abrupt and gradual turners. In view of the lack of species differences in either measure, no distinction was drawn between woodlice and pillbugs for this analysis. While neither correlation was significant for abrupt turners [pre/angle, $r(28) = .16$; post/angle, $r(28) = -.22$], both were significant for gradual turners [pre/angle, $r(64) = -.25$, $p < .05$; post/angle, $r(64) = -.38$, $p < .01$].

Since abrupt turners of both species traveled closer to the outer wall before as well as after the forced turn and then turned at sharper angles than gradual turners, it was possible that their free-choice behavior had

been at least partly determined by the amount of tactile stimulation experienced while following the two walls. This was supported by the small (but significant) negative correlation between free-turn angle and distance from the start-alley outer wall for gradual turners indicating that the closer subjects were to this wall the sharper they tended to subsequently turn. While a similar relationship observed for exit-alley behavior could be attributed to BALM effects (Hughes, 1985), this explanation would not account for the start-alley finding since no prior turning had occured. The lack of significant correlations between those two measures for abrupt turners was probably due to ceiling (or wall!) effects arising from their much closer proximity to both outer walls. Abrupt turns were probably the inevitable result of restricted movement caused by traveling close to the start-alley outer wall. Overall the results suggested involvement of a graded responsiveness to tactile cues on the angle finally turned.

EXPERIMENT 2

In the next experiment, the effects of tactile stimulation (arising from contact with a vertical surface) on turning behavior were further investigated. If a graded responsiveness to tactile cues can influence the angle turned, the length of and thus duration of contact with a vertical surface should determine the angle turned on emerging from a straight runway. Isopods are thigmotactic (Pardi & Papi, 1961) and will turn in the same direction as a followed wall without having encountered any prior forced turn (Hughes, 1987).

Method

Animals and Apparatus.—The subjects were 40 woodlice and 40 pillbugs. The apparatus comprised two straight 10-mm-wide, 6-mm-thick clear Perspex runways. One was 60 mm long and the other was 180 mm long. As for Experiment 1, a series of parallel lines intersected by other lines at 10-mm intervals were drawn on the floor of each runway. Each exit was positioned in the centre of a circle and a TV camera and video-recorder was used to record runway behavior.

PROCEDURE

Twenty members of each species were individually introduced into the closed end of each runway for a single trial. Progress along the runway was video-recorded. On emerging from the exit, the direction turned and the point crossed on the circumference of the circle were noted. The video-record was later replayed and images frozen at 40, 30,

20, 10 and 0 mm from the exit to enable measurements of average distances from the wall on the same side as the direction subsequently turned.

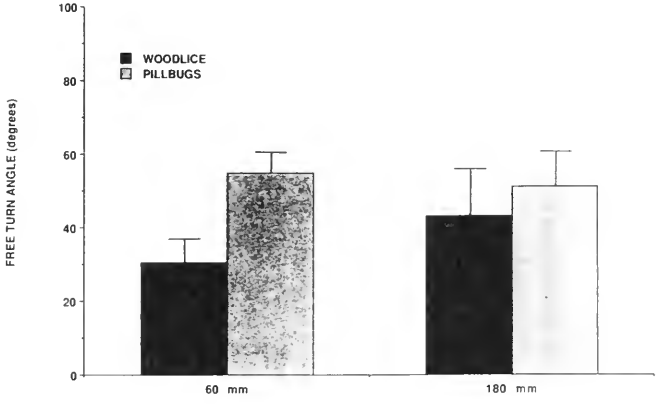

FIGURE 3

Mean + SE free-turn angle for woodlice and pillbugs emerging from 60- and 180-mm-long straight runways.

RESULTS AND DISCUSSION

Angles turned by each species after emerging from the two runways can be seen in Figure 3. While pillbugs emerged at sharper angles than woodlice [$F(1, 76) = 4.44$, $p < .05$], runway length did not affect turning behavior [$F(1, 76) < 1$] nor was the Species X Runway length interaction significant [$F(1, 76) = 1.17$].

Mean $\pm$ SEM average distances (in mm) from the wall on the same side as the direction turned after emerging from the 60- and 180-mm runways respectively were, for woodlice, $2.54 \pm .21$, $2.05 \pm .20$, and for pillbugs, $2.43 \pm .26$ and $2.35 \pm .19$. Neither species [$F(1, 76) < 1$] nor runway length [$F(1, 76) = 1.68$] or their interaction affected this measure [$F(1, 76) < 1$]. However, there was a small significant negative correlation between average distance from the followed wall and free-turn angle for woodlice in both runways combined [$r(38) = .35$, $p < .05$] but not for pillbugs [$r(38) = -.07$]. This suggested that the closer woodlice were to the wall, the sharper were their subsequent turns in its direction.

The lack of any relationship between wall length and free-turn angle for either species does not support any influence of a graded responsiveness to tactile cues on free-turn angle in the manner suggested for centipedes by Schäfer (1976). On the other hand, the negative correlation between distance from the followed wall and free-turn angle for

woodlice suggests, for this species, a weak quantitative relationship between tactile stimulation and turning behavior which is, however, independent of duration of such stimulation.

The sharper angles turned by pillbugs compared with woodlice cannot be readily accounted for apart from speculating that slight species differences in body shape or leg size and orientation were in some way responsible. *E. purpurascens* is narrower and less dorsoventrally flattened with shorter legs than *P. scaber*. Such factors might also account for the species difference in the relationship between average distance from a followed wall and free-turn angle.

EXPERIMENT 3

Because of the species differences observed in Experiment 2, it seemed desirable to partially replicate the study paying particular attention to possible quantitative relationships between distances from a followed wall and free-turn size. In order to achieve more accurate averages, distances from the wall were sampled at a greater number of points along the runway. Since it was suspected that, during each Experiment 2 trial, pillbugs but not woodlice gradually moved closer to the followed wall while progressing towards the exit, changes in this measure from the beginning to the end of the runway were also assessed.

Method

Thirty woodlice and 30 pillbugs were observed in a 120-mm-long runway. Other dimensions, means of measuring free-turn angles and video-recording equipment were the same as in Experiment 2. The procedure was the same as in Experiment 2 except that, when played back, video-recorded images were frozen at 9 points 10 mm apart ranging from 90 to 0 mm from the exit.

RESULTS AND DISCUSSION

The mean $\pm$ SE free-turn angle was $29.57 \pm 5.03°$ for woodlice and $45.77 \pm 5.21°$ for pillbugs. This difference was significant [$t(58) = 2.24$, $p < .05$].

For each subject, average distances from the wall on the same side as the direction turned were calculated for points 80-60, 50-30 and 20-0 mm from the exit. The results for each species can be seen in Figure 4. Whereas the species effect was not significant [$F(1, 58) < 1$], there were significant differences between the points where measurements were taken [$F(2, 116) = 3.21$, $p < .05$]. However, this effect is more appropriately considered in the light of the significant interaction between

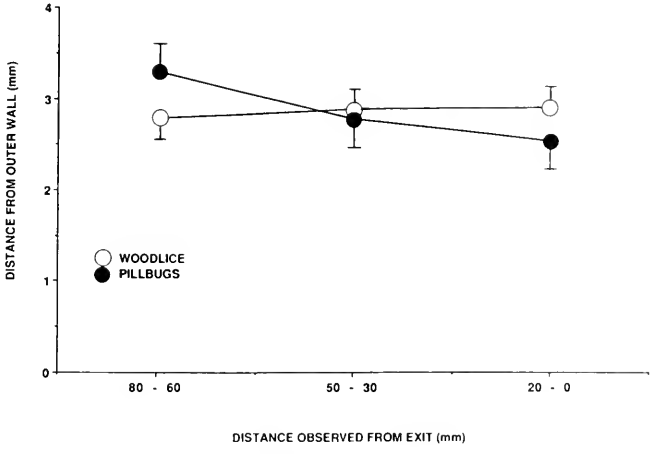

FIGURE 4

Mean + SE distance from the followed (outer) wall observed at three distances from the exit of a straight runway for woodlice and pillbugs.

the two factors [$F(2, 116) = 6.16, p < .01$] thereby confirming the obvious tendency outlined in Figure 4 for pillbugs but not woodlice to move closer to the followed wall as the exit was approached.

Average distances from the followed wall at 80-60, 50-30 and 20-0 mm from the exit were correlated with free-turn angle for each species separately. The results can be seen in Table 1. The only significant correlation was for woodlice when observed 20-0 mm from the exit.

As in Experiment 2, pillbugs turned at sharper angles than woodlice. The difference in free-turn angle between the two experiments was not significant for either species [$t(68) < 1$ in both cases]. The tendency for pillbugs but not woodlice to move closer to the followed wall as the

Table 1
Correlations Between Average Distances from the Followed Wall and Free-turn Angle for Woodlice and Pillbugs

	Coefficients [r(28)] for distances (mm) from exit		
Species	*80-60*	*50-30*	*20-0*
Woodlouse	-.05	-.10	-.43*
Pillbug	-.08	-.10	-.24

*$p < 0.05$

exit was approached reflected slight differences in locomotor styles. It was evident that both species began their runs with an obvious yawing motion but as they moved closer to the exit this pattern changed to a more stable attachment to the wall for pillbugs only, thereby producing shorter average distances for this species. This more consistent tactile stimulation for pillbugs might have accounted for their sharper free turns, in terms of a quantitative relationship between such stimulation and turning behavior, if it were not for the lack of any relationship between the angles they turned and their distances from the followed wall. On the other hand, the closer individual woodlice were to this wall when 20, 10 or 0 mm from the exit, the sharper were their subsequent turns.

However, a graded responsiveness to tactile cues seemed of minimal importance in determining the size of a subsequent free turn because of the ineffectiveness of wall length on free-turn angle, the nonsignificant distance-from-wall/free-turn-angle correlations for pillbugs and the lack of any relationship between the two measures for woodlice when further than 20 mm from the exit. Added to this is the small amount of variance accounted for by the two significant correlations for woodlice in Experiments 2 and 3, namely 15.21% and 18.49% respectively. It is therefore likely that the differences between abrupt and gradual turners observed in Experiment 1 were mainly due to BALM effects rather than to differing amounts of tactile stimulation. By initially traveling closer to the start-alley outer wall, abrupt turners were more likely to collide with the exit-alley outer wall than gradual turners thereby necessitating sharper forced turns. The generation of bilateral asymmetry in leg movements would have obviously been greater for abrupt turners thus causing them to travel closer to the exit-alley outer wall and emerge at more acute angles.

EXPERIMENT 4

Schäfer (1982) has proposed that the storage of information in isopods about previous turns (which is later used in determining the size of a free-turn) arises from the distortion of body segments. If such a mechanism rather than BALM effects were responsible for alternation, varied demands on different legs should have no effect on free-turn angle provided body distortion is kept constant. The final experiment aimed to distinguish between the two explanations.

It was reasoned that lining the outer half of a runway with smooth glass in the vicinity of a 90° forced turn should cause subjects' inner legs to grip a rougher surface more firmly than the outer thereby exerting proportionately more locomotor force than normal during the negotiation of a turn. Bilateral asymmetry in leg activity should be less and

free-turn angles smaller than if glass were not present because of the greater relative influence of outer legs worked less than normal. However, if distortion of body segments determined alternation, the presence of glass should have no effect since, while interferring with normal leg movements during turning, distortion would be the same whether or not glass were present.

Method

Subjects and Apparatus.—The subjects were 48 woodlice no narrower than 5 mm. (Pillbugs were not used since the nature of the experimental manipulation did not suit their shorter legs.) The runway was the same as for Experiment 1 except that, for half the subjects, the outer half of the paper floor was lined with 0.2-mm-thick glass from 27 mm before to 30 mm past the forced turn. The same video-recording equipment was used as in previous experiments.

PROCEDURE

Subjects were given one trial in the runway either with or without glass being present. For each animal, its progress along the exit alley was video-recorded, its free-turn angle noted and its video-record later replayed with images frozen at 30, 20, 10 and 0 mm from the exit to enable measurements of distance from the outer wall. Half the woodlice exposed to each type of floor were forced left and half were forced right.

RESULTS AND DISCUSSION

Some woodlice did not travel along the runway with legs from one side of the body consistently on glass and those from the other side on paper, while others turned around in the exit alley before emerging. Consequently data from 8 subjects exposed to glass (which appeared aversive) and 4 run on paper alone were excluded from statistical analyses.

Mean $\pm$ SE free-turn angles for woodlice tested with and without glass were $17.13 \pm 10.55°$ and $47.70 \pm 10.35°$ respectively. The difference was significant [$t(34) = 2.07$, $p < .05$]. Only 11 of the 16 subjects (69%) run on glass alternated [$p < .2$] whereas 16 out of the 20 (80%) run on paper alone alternated [$p = .012$]. For woodlice run on either glass or paper alone, mean $\pm$ SE average distances from the exit-alley outer wall were $2.95 \pm .17$ and $2.07 \pm .17$ mm respectively. The difference was significant [$t(34) = 3.64$, $p < .01$].

For reasons outlined earlier, it seems likely that the presence of glass lessened bilateral asymmetry in leg movements during the forced turn.

The greater distances from the exit-alley outer wall shown by woodlice run on glass indicates that their legs on this side had more relative influence on free-turn behavior than when glass was not present. Consequently, the results of this experiment are consistent with BALM effects rather than distortion of body segments being responsible for the size of free turns in the opposite direction to a forced turn.

GENERAL DISCUSSION

From the results of Experiments 2 and 3 it is clear that while tactile cues might determine the direction of a turn, they have minimal effects on its size in isopods. It is particularly notable that, following the results of these two experiments, enhancement of alternation by seemingly graded thigmotaxis-based wall following in Experiment 1 was later more satisfactorily explained by relationships between distances initially traveled from the start-alley outer wall and the nature of the subsequent turn required to move into the exit alley. Although at times under unusual circumstances they may replace the operation of proprioceptive mechanisms (Hughes, 1987), tactile stimuli are unlikely to play a significant part in determining the size of most alternating turns in the two isopod species investigated.

The results of Experiment 4 support the involvement of proprioceptive feedback from BALM effects (Hughes, 1985) rather than body distortions (Schäfer, 1982) in woodlouse alternation. However, it is unlikely that BALM effects alone will account for all cases of alternation since isopods still alternate when passively moved around a forced turn without leg movementis (Schäfer, 1986). Although the amputation of one or both antennae does not affect isopod turn alternation (Hughes, 1978, 1985; Schäfer, 1986), Heggemann and Wendler (1981) have implicated asymmetrical stimulation of the antennae in the coordination of leg movements. It is therefore likely that no single mechanism is crucial. Depending on circumstances, BALM effects, body distortions, activity of the antennae and tactile cues may all contribute to the control of alternation but with proprioceptive feedback probably playing the major role. To avoid dessication and predators, woodlice generally prefer to inhabit damp, protected environments (Sutton, 1972). If forced out into the open they may have to travel considerable distances as quickly as possible before encountering another suitable habitat. A combination of thigmotaxis and centrifugal swing is sufficient to maintain a pattern of alternating turns at successive physical objects. However, an ability to alternate by means of proprioceptive cues would be necessary for negotiating nontactile obstacles such as areas of dryness, heat or chemical contamination. Instead of this type of mechanism operating only in the absence of tactile cues, results of the present and earlier

studies (Hughes, 1985; Kupfermann, 1966) indicate its predominant importance for isopods negotiating turns both with and without tactile stimulation.

Provision of glass at the forced turn was more likely to have modified differences in effort exerted by legs on each side of the body rather than distance traveled during the forced turn. Accordingly, it was possible that a fatigue-related process was involved in the BALM effects, as suggested earlier (Hughes, 1985). However, it is maladaptive and thus unlikely for the large bilateral differences in fatigue that would be required for the production of an alternating turn to be generated by a single forced turn. It is also improbable that such differences would rapidly dissipate with continued effort as suggested by decreases in alternation with greater distances between forced and free turns (Hughes, 1967; Schäfer, 1982). Instead, it is more likely that isopod alternation mediated by leg movements arises from proprioceptive feedback from the legs to sites in the central nervous system responsible for the integration of inhibitory and excitatory influences in the control of turning behavior. In view of the elaborate array of proprioceptors found in the various joints of the crustacean limb and their importance in every aspect of movement (Evoy & Ayers, 1982), more detailed examinations of leg movements during turning at forced and free turns are clearly indicated.

ACKNOWLEDGEMENTS

This research was supported by University of Canterbury research grant number 576761. The assistance of Peter M. Johns in the identification of isopod species is gratefully acknowledged.

REFERENCES

Barnwell, F. H. (1965). An angle sense in the orientation of a millipede. *Biol. Bull., 128,* 33-50.

Beale, I. L. & Webster, D. M. (1971). The relevance of leg-movement cues to turn alternation in woodlice *(Porcellio scaber). Anim. Behav., 19,* 353-356.

Dingle, H. (1964). Further observations on correcting behaviour in boxelder bugs. *Anim. Behav., 12,* 116-124.

Evoy, W. H. & Ayers, J. (1982). Locomotion and control of limb movements. In D. D. Sandeman & H. L. Atwood (Eds.), *The Biology of Crustacea* Vol. 2, (pp. 61-105). New York: Academic Press.

Heggemann, B. & Wendler, G. (1981). Analyse des Gegenwendeverhaltens bei Landasseln. *Verh. Dtsch. Zool. Ges., 74,* 233.

Hughes, R. N. (1967). Turn alternation in woodlice *(Porcellio scaber). Anim. Behav., 15,* 282-286.

Hughes, R. N. (1978). Effects of blinding, antennectomy, food deprivation and simulated natural conditions on alternation in woodlice *(Porcellio scaber). J. Biol. Psychol., 20,* 35-40.

Hughes, R. N. (1985). Mechanisms for turn alternation in woodlice *(Porcellio scaber):* The role of bilaterally asymmetrical leg movements. *Anim. Learn. Behav., 13,* 253-260.

Hughes, R. N. (1987). Mechanisms for turn alternation in four invertebrate species. *Behav. Proc., 14,* 89-103.

Hughes, R. N. (1989). Phylogenetic comparisons. In W. N. Dember & C. L. Richman (Eds.), *Spontaneous Alternation Behavior,* (pp. 39-57). New York: Springer-Verlag.

Hurley, D. E. (1950). New Zealand terrestrial isopoda. *Tuatara, 3,* 115-127.

Iwata, K. & Watanabe, M. (1957). Alternate turning response of *Armadillidium vulgare.* II. Turning and straight going tendencies. *Ann. Anim. Psychol., 1,* 53-57.

Jans, D. E. & Ross, K. F. A. (1963). A historical study of the peripheral receptors in the thorax of land isopods, with special reference to the location of possible hygroreceptors. *Quart. J. Micr. Sci, 104,* 337-350.

Johns, P. M. (1989). *The Terrestrial Isopods of New Zealand.* Zoology Department, University of Canterbury.

Kupfermann, I. (1966). Turn alternation in the pill bug *(Armadillidium vulgare). Anim. Behav., 14,* 68-72.

Pardi, L. & Papi, F. (1961). Kinetic and tactic responses. In T. H. Waterman (Ed.), *The physiology of crustacea,* Vol. 2, pp. 365-399. New York: Academic Press.

Richman C. L., Dember, W. N. & Kim, P. (1987). Spontaneous alternation behavior in animals: A review. *Curr. Psychol. Res. Rev., 5,* 358-391.

Schäfer, M. W. (1972). Reverse turning in Lithobius forficatus L. *Monitore Zool. Ital., 6,* 179-194.

Schäfer, M. W. (1976). Thigmotactic behaviour in Lithobius forficatus L. (Myriapoda Chilopoda). *Monitore Zool. Ital., 10,* 191-204.

Schäfer, M. W. (1982). Ein idiothetischer Mechanismus im Gegendrehungsverhalten der Assel *Oniscus asellus* L. *Zool. Jb. Physiol. 86,* 193-208.

Schäfer, M. W. (1986). Erganzende Untersuchungen zum Gegendrehungsverhalten der Asseln *Oniscus asellus* L. und *Porcellio scaber* LATR. *Zool. Jb. Physiol., 90,* 285-296.

Sutton, S. (1972). *Woodlice.* London: Ginn & Co.

The International Journal of Comparative Psychology, Vol. 2, No. 4, Summer 1989

SOCIAL INFLUENCES ON THE FOOD PREFERENCES OF HOUSE MICE *(MUS MUSCULUS)*

Paola Valsecchi
Universita di Parma

Bennett G. Galef, Jr.
McMaster University

ABSTRACT: In a series of studies undertaken to determine the conditions under which naive house mice (observers) develop preferences for foods eaten by recently-fed conspecifics (demonstrators), we found that observer mice exhibited enhanced preference for a food following interaction with either a healthy or an ill recently-fed demonstrator that had eaten that food. We also found that house mice developed an enhanced preference for a food after exposure to an anesthetized conspecific demonstrator powdered with that food, but not after exposure to a cotton-batting, conspecific-sized surrogate powdered with the same food. Results of other studies have indicated that, for both rats and mice, the presence in a food of carbon disulfide (a substance found on the breath of rats) increases preference for a carbon-disulfide-contaminated food. Taken together, the parallels between Norway rats and house mice in social learning processes suggest homologous rather than analogous systems of communication about distant foods in these two murid rodents.

During social contact between a recently-fed Norway rat (a demonstrator) and a naive conspecific (an observer), olfactory cues pass from demonstrator to observer increasing the observer's subsequent preference for the food its demonstrator ate (Galef & Wigmore, 1983; Posadas-Andrews & Roper, 1983). Studies carried out in our laboratory during the past 5 years have provided a detailed picture of both the social interactions and chemical signals responsible for this social influence on diet choice in domesticated rats (For reviews see Galef 1988, 1989a). The experiments described here were undertaken to determine whether the same behavioral processes that support social transmission of information about distant foods in Norway rats *(Rattus norvegicus)* might be found in a second species of myomorph rodent, *Mus musculus.*

Address all correspondence to Dr. Paula Valsecchi, Instituto di Zoologia, Universita di Parma, 43100 Parma, Italy.

House mice, like Norway rats, are members of the subfamily Murinae, the Old World rats and mice. Outside the laboratory, both Norway rats and house mice are social animals, both are dietary generalists, and both are cosmopolitan, human commensals, subjected to human-introduced poisons in many parts of their largely-overlapping species ranges (Nowak & Paradiso, 1983). Most relevant to the present studies, both Norway rats and house mice are social, central-place foragers (Ward & Zahavi, 1973). Members of each species live in fixed home sites from which they emerge to forage and to which they return periodically. Further, because members of both species interact with conspecifics at their respective home sites, they have the opportunity to exploit conspecifics as sources of information about distant foods.

Thus, on both phylogenetic and ecological grounds, one might expect Norway rats and house mice to exhibit similar effects of social influence on their feeding behaviors. In particular, given that rats use conspecifics as sources of information about what foods to eat (Galef, 1989a; Galef & Wigmore, 1983), one might predict that house mice would do so as well. It is not, however, at all clear whether one should expect such phenotypic similarity to extend from overt behavior to underlying process.

If rats and mice share only a tendency to eat what others of their species are eating, then such similarity in behavior might well be a convergent response to similar ecological demands rather than the result of homologous social learning process. On the other hand, if details of the learning processes involved in social transmission of food preferences were identical in rats and mice, it would suggest that social learning about foods was homologous in the two species (Simpson, 1961). The experiments described below were undertaken to determine whether the details of the processes of social influence on food choice of house mice were similar to the processes of social influence on food choice exhibited by Norway rats.

EXPERIMENT 1

In previous experiments concerned with social learning about distant foods by Norway rats (see for example Galef & Wigmore, 1983) food-deprived demonstrator rats were fed either a cocoa- or a cinnamon-flavored diet for 30 min. Each demonstrator was then allowed to interact with an experimentally-naive observer rat for 15 min. Later, when observer rats were offered a choice between cinnamon- and cocoa-flavored diets, they exhibited a robust preference for whichever of the two diets their respective demonstrators had eaten. In the present experiment, we repeated this basic procedure using domesticated house mice rather than domesticated Norway rats as subjects. Our goal was to

determine whether mice, like rats, would use conspecifics as sources of information about which food to eat.

Method

Animals. — Sixteen experimentally-naive, adult female albino mice *(Mus musculus)* of the CD-1 strain (obtained from Charles River Canada, St. Constant, Quebec), weighing 20-25 g, served as observers. Sixteen additional, similar females served as demonstrators.

All animals were housed and tested in temperature- and humidity-controlled animal rooms maintained on a 12-hr light/dark cycle (light onset at 0700 hr). Interactions between demonstrators and observers (see Procedure) were initiated between 1045 and 1100 hr.

Apparatus. — Subjects were housed in demonstrator-observer pairs in 30 x 30 x 15 cm stainless-steel home-cages. Each home-cage was divided into two equal parts by a double-walled, opaque partition. Individual observers were tested for their food preferences in a 32.5 x 37.5 x 16.5 cm plastic, shoebox cage, covered with a 1/4-in. (.62-cm) hardware cloth lid.

To permit precise measurement of food intake, food was presented to both demonstrators and observers in specially-designed feeding devices. Each feeding device was constructed by attaching a 4.5 x 4.5 cm glass jar with a Bakelite lid (in which a 2-cm-diameter hole had been drilled) to the center of a 4 x 8-cm-diameter, Pyrex crystallizing dish (Corning Glass, Corning, NY). The small opening in the food jar reduced spillage of the powdered food each jar contained and any spillage that did occur was almost always trapped in the surrounding Pyrex dish. Data were discarded from two animals that spilled food outside the feeding device.

PROCEDURE

Experiment 1 was conducted in five steps:

Step 1. — Each demonstrator-observer pair was placed together in the same compartment of a home-cage and maintained ad lib on pellets of Purina Laboratory Rodent Chow and water for a 2-day period of familiarization with both partner and apparatus.

Step 2. — To ensure that demonstrators ate when they were given the opportunity to do so, the demonstrator in each pair of subjects was moved in its home-cage to the opposite side of the double partition from its observer and was food deprived for 24 hr.

Step 3. — At the end of this 24-hr period of food deprivation, a weighed feeding device containing either a cocoa-flavored diet (Diet Coc: powdered Purina Laboratory rodent Chow adulterated 2% by weight with sifted Hershey's Pure cocoa) or a cinnamon-flavored diet (Diet Cin: powdered Purina Laboratory Rodent Chow adulterated 1% by weight with McCormick's Fancy Ground Cinnamon) was presented to each demonstrator for 45 min. While demonstrators were eating, the experimenter removed the food from each observer's side of the home-cage. At the end of the 45-min demonstrator feeding period, the experimenter weighed each feeding device on a balance sensitive to 0.1 g.

Step 4. — Each demonstrator was moved back to the side of the home-cage containing its observer pair-mate and demonstrator and observer were allowed to interact freely for 30 min.

Step 5. — Demonstrators were removed from the experiment and observers were placed in the individual, shoe-box, test cages described in *Apparatus.* Each test cage contained two weighed feeding devices, one holding Diet Cin and one holding Diet Coc. Observers were left undisturbed for 24 hr to eat from the two feeding devices.

At the end of this 24-hr test period, the experimenter weighed the feeding devices on a digital balance accurate to 0.1 g and calculated the percentage of each observer's total intake eaten from the feeding device containing Diet Cin.

RESULTS AND DISCUSSION

The results of Experiment 1 are presented in Figure 1, which shows the mean amount of Diet Cin eaten by observers during the 24-hr test period, as a percentage of observers' total intakes during testing. As can be seen in Figure 1, those observers whose demonstrators ate Diet Cin ate considerably more Diet Cin than did those observers whose demonstrators ate Diet Coc (Mann-Whitney U test, $U = 3$, $p < .005$). These results demonstrate that mice, like rats (Galef & Wigmore, 1983), can be influenced in their diet choices by information extracted during a brief period of interaction with recently-fed conspecifics.

An unexpected finding in previous studies of social effects on diet selection by Norway rats is that observer rats developed preferences not only for foods eaten by healthy conspecific demonstrators but also for foods eaten by obviously-ill conspecific demonstrators (Galef, Wigmore & Kennett, 1983). Rats appear to learn from conspecifics what foods to eat, but not what foods to avoid eating. In the present experiment, we determined whether observer mice would prefer or avoid a food eaten by an obviously-ill, conspecific demonstrator with which they interacted.

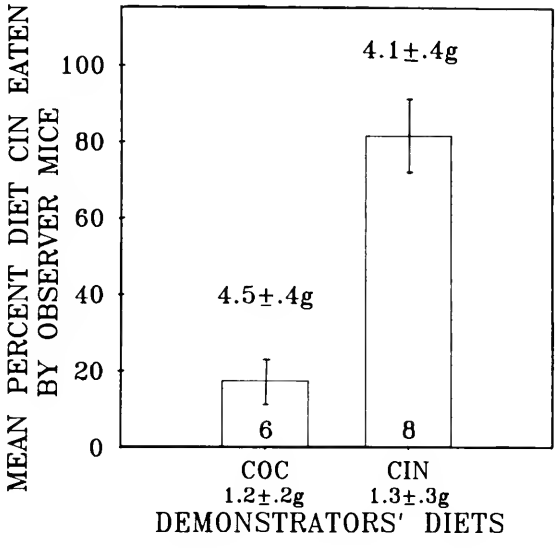

Figure 1

Mean amount of Diet Cin eaten by observers during testing (Step 5), as a percentage of total amount eaten. Bars indicate ± 1 SE; digits in histograms = N/group; numbers above histograms = mean g (± 1 SE) of diet eaten by observers during Step 5 of Procedure. Numbers below abscissa = mean g (± 1 SE) of diet eaten by demonstrators during Step 3.

EXPERIMENT 2

Method

Animals. — Thirty-six experimentally-naive, adult female mice of the CD-1 strain, similar to those used in Experiment 1, served as observers in the present experiment. An additional 36 mice that had served as observers during the previous 2 weeks served as demonstrators.

Apparatus. — The same apparatus was used as in Experiment 1.

PROCEDURE

The procedure was identical to that of Experiment 1 except that immediately following feeding of demonstrators (Step 3 of Procedure), each demonstrator was injected intraperitoneally with either 0.7 cc of .24 M lithium-chloride (LiCl) solution or 0.7 cc of physiological saline. Pilot studies has shown that intraperitoneal injection with such a dose of LiCl produced profound learned aversions in 20-25 g female mice. Adult female CD-1 mice fed Diet Cin and then injected with 0.7 cc of .24 M LiCl solution became lethargic, had severe and conspicuous diarrhea, and

when offered a choice between Diet Cin and Diet Coc 24 hr after injection, ate only Diet Coc.

In the present experiment, half the demonstrators fed Diet Cin and half the demonstrators fed Diet Coc during Step 3 were injected with LiCl solution just before they interacted with their respective observers (Step 4). The other half of each group of demonstrators were injected with saline solution between Steps 3 and 4 of Procedure.

RESULTS AND DISCUSSION

The results of Experiment 2 are presented in Figure 2 which shows the mean percent of Diet Cin eaten during testing by observers that interacted with either saline- or LiCl-injected demonstrators that had eaten either Diet Cin or Diet Coc. As can be seen in Figure 2, during testing, both observers that interacted with LiCl-injected demonstrators and observers that interacted with saline-injected demonstrators showed a marked preference for the diet eaten by their respective demonstrators (Mann-Whitney *U* tests; see figure 2 for *U* and *p* values). As is also evident from inspection of Figure 2, the magnitude of the effects of demonstrators on the food preferences of their respective observers was not affected by the state of health of those demonstrators during the period when demonstrators and observers interacted. Food choices of observer mice, like those of observer rats, were influenced by

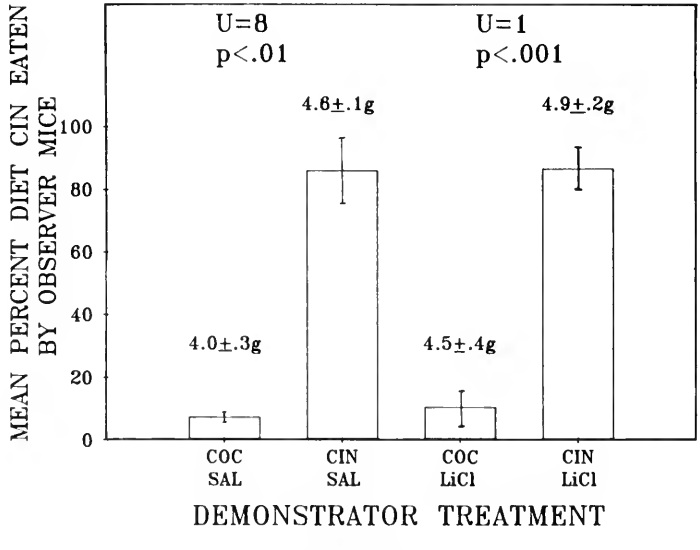

Figure 2

Mean amount of Diet Cin eaten by observers during testing (Step 5) as a percentage of total amount eaten. Bars indicate ± 1 SE; numbers above histograms = mean g (± 1 SE) eaten by observers during testing (Step 5). N = 9 observers in each group.

the foods eaten by conspecific demonstrators and not by the health or illness of those demonstrators.

EXPERIMENT 3

The results of previous studies of Norway rats have shown that olfactory signals passing from recently-fed Norway rat demonstrators to their conspecific observers cause the observers to exhibit enhanced preferences for the foods eaten by their respective demonstrators (Galef & Wigmore, 1983). Our data have also indicated that although simple exposure to the taste or smell of a food is often insufficient to enhance the preferences of naive rats for that food, exposure to the same food sprinkled on the face of a demonstrator is sufficient to enhance naive rats' preferences for that food (Galef, 1989b; Galef, Kennett & Stein, 1985; Galef & Stein, 1985).

The present experiment was undertaken to discover whether, for mice as for rats: (a) olfactory cues passing from demonstrator to observer are sufficient to permit demonstrator influence on observer diet preference and (b) the presence of a conspecific demonstrator renders exposure to a food more effective in altering observers' later diet preferences than equivalent exposure to the same food in the absence of a conspecific demonstrator.

Method

Animals. — In the present experiment, 72 experimentally-naive, 20-25 g, female, albino mice of the CD-1 strain served as observers. An additional 52 similar 32-35 g mice, that had been subjects in other experiments, served as demonstrators.

Apparatus. — The apparatus used in the present experiment was the same as that used in Experiments 1 and 2 except during Step 4, the period of interaction of demonstrators and observers. In the present experiment, each demonstrator-observer pair interacted for 30 min in an apparatus constructed from a 2.45 liter (15.2 cm high, 19.0 cm top diam., 14.0 cm bottom diam.) cardboard bucket (Lily-Tulip Inc., Toledo, OH) of the type used by many fast-food franchises. A circular opening (5 cm diam.) was cut in the side of the bucket 12 cm above its floor. Through this opening a cylindrical tube of 1/4-in. (.63-cm) hardware cloth (16 cm long, 5 cm diam.) was inserted for half its length. The end of the cylindrical tube inside the bucket was closed with 1/4-in (.63 cm) hardware cloth; the end outside the bucket was left open. Cardboard lids prevented observers from leaving their respective buckets (See Figure 4 in Galef, Kennett & Stein, 1985, for an illustration of the apparatus).

PROCEDURE

Steps 1, 2, and 5 in the present experiment were identical to the same steps in Experiments 1 and 2. However in the present experiment, observers interacted with demonstrators during Step 4 in the apparatus described immediately above rather than in their respective home-cages. Further, during Step 3, the demonstrators with which observers interacted during Step 4 were treated in a variety of different ways described below.

Fed-Demonstrator Group (16 observers and 16 demonstrators). — Each demonstrator assigned to the Fed-Demonstrator Group (Fed-Dem Group) was fed either Diet Cin (n = 8) or Diet Coc (n = 8) for 45 min (Step 3) and was then anesthetized by intraperitoneal injection (60 mg/kg sodium pentobarbitol). Each anesthetized demonstrator was placed in a cylindrical, hardward-cloth tube (with its head inside the apparatus) for 30 min to interact with an observer placed in the cardboard bucket.

Powdered-Demonstrator Group (20 observer and 20 demonstrators). — Each demonstrator assigned to the Powdered Demonstrator Group (Powdered-Dem Group) group was not fed during Step 3, but was, instead, anesthetized and had its muzzle powdered with either Diet Cin (n = 10) or Diet Coc (n = 10). Each demonstrator was then placed in a cylindrical, hardware-cloth tube to interact with an observer placed in the cardboard bucket for 30 min.

Powdered-Demonstrator-on-a-Platform Group (18 observers and 18 demonstrators). — Demonstrators assigned to the Powdered-Demonstrator-on-a Platform-Group (Powdered-Platform Group) were treated identically to those assigned to the Powdered-Dem Group described above except that each demonstrator in the Powdered-Platform Group was fixed with a strip of adhesive tape to a 5 x 16-cm, rectangular piece of plastic that, when placed in the cylindrical, hardware-cloth tube of the apparatus, made impossible direct physical contact between demonstrator and observer during Step 4.

Surrogate-Demonstrator Group (20 observers). — During Step 4, observers assigned to the Surrogate-Demonstrator Group (Surrogate-Dem Group) interacted not with an anesthetized, powdered mouse, but with a mouse-sized, cotton-batting, surrogate mouse one end of which had been powdered with either Diet Cin (n = 10) or Diet Coc (n = 10). Surrogates were constructed by stuffing an appropriate amount of cotton batting into a length of tubular gauze (Size 12 Tubegauz, School Canada Inc., Toronto, Ont.) and stapling one end of the gauze tube

closed. Surrogate demonstrators were introduced into the cylindrical, hardware-cloth tube with the powdered, closed end inside the bucket and were left there throughout Step 4 of Procedure.

RESULTS AND DISCUSSION

The results of Experiment 3 are presented in Figure 3 which shows the mean percentage of Diet Cin eaten during testing by observers in the various groups.

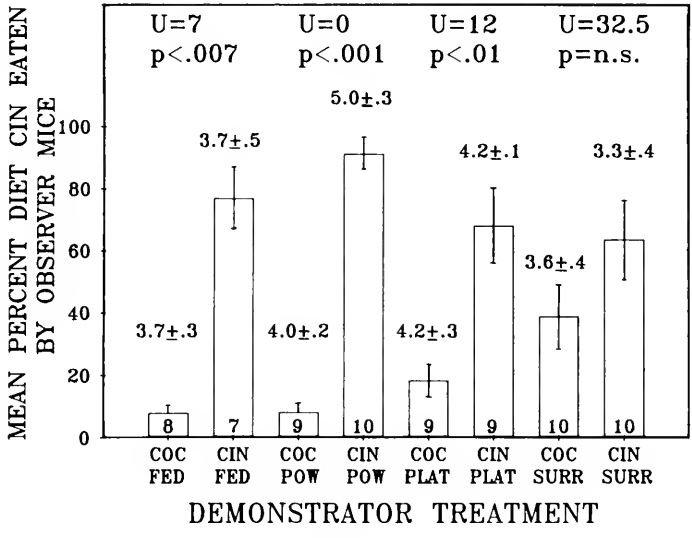

Figure 3

Mean amount of Diet Cin eaten by observers during testing (Step 5) as a percentage of total amount eaten. Bars indicate ± 1 SE; digits in histogram = N/group; numbers above histograms = mean g (± 1 SE) eaten by observers during testing (Step 5).

As can be seen in Figure 3, the diets fed to or powdered on demonstrators in Fed-Dem, Powdered-Dem, and Powdered-Platform Groups had profound effects on the later diet preferences of their respective observers. In each case, those observers interacting with demonstrators fed or powdered with Diet Cin exhibited a greater preference for Diet Cin than did those observers interacting with demonstrators fed or powdered with diet Coc (Mann-Whitney U tests, see Figure 3 for U and p values). As can also be seen in Figure 3, interaction of an observer mouse with a powdered, surrogate demonstrator was less effective in altering observers' diet preferences than was interaction with a powdered, anesthetized demonstrator. The diet powdered onto a surrogate failed to significantly effect observers' subsequent diet preferences.

Several conclusions can be drawn from the results of this experiment. First, the finding that anesthetized demonstrator mice can influence the diet preferences of their respective observers indicates that in mice, as in rats, signals coming from demonstrators that influence an observer's later diet choices are passively emitted by demonstrators rather than elicited by observers.

Second, the observation that demonstrator mice powdered with a diet, rather than fed a diet, affected their observers' later food choices leads to the conclusion that in mice, as in rats, it is not necessary for a demonstrator to eat a food to influence the later food preferences of its observer.

Third, the fact that physical contact between demonstrator and observer was not necessary for induction of changes in the diet preferences of observer mice suggests that in mice, as in rats, olfactory cues emitted by demonstrators suffice to influence the food preferences of their respective observers.

Finally, we found that in mice, as in rats, exposure to a diet powdered on an anesthetized demonstrator, but not to a diet powdered on cotton-batting surrogate, was effective in altering observers' later diet preferences. In both rats and mice the presence of a conspecific appears to be critical in causing exposure to a diet to influence observers' food choices.

GENERAL DISCUSSION

The results of the present series of experiments demonstrate a number of parallels between the processes underlying social influences on diet choice in rats and in mice. Both naive rats and naive mice prefer to eat foods that conspecifics have eaten (Experiment 1); neither avoids foods that ill (Experiment 2) or unconscious (Experiment 3) conspecific have eaten. Both rats and mice are influenced in their later food choice by the food powdered on a demonstrator, but not by the same food powdered on a cotton surrogate (Experiment 3). Neither rats nor mice require physical contact with a diet-powdered demonstrator to be influenced by it (Experiment 3). Finally, we know from other research that carbon disulfide (a chemical found in rat breath) when added to a food, increased the attractiveness of that food to both rats and mice (Bean, Galef, & Mason, 1988; Galef, Mason, Preti & Bean, 1988; Mason, Bean & Galef, 1989).

Simpson (1961) has proposed that in seeking to identify homologous structures (i.e., those phenotypic characteristics that are similar as the result of descent from a common ancestor) multiplicity of similarities and minuteness of resemblances are important criteria. To date we

have found no differences in the details of either the behavioral processes or chemical signals that support social transmission of diet preference in rats and mice. These findings are consistent with the view that homologous mechanisms operate in social learning about distant foods in two central-place-foraging, generalist, murid rodents, *Rattus norvegicus* and *Mus musculus*.

ACKNOWLEDGEMENT

The research reported here was supported both by grants from the Natural Sciences and Engineering Research Council of Canada and the McMaster University Research Board to Bennett G. Galef, Jr. and by an Italian Ministry of Education grant to Paola Valsecchi.

We thank Mertice Clark for her thoughtful critique of earlier drafts of the present manuscript.

REFERENCES

Bean, N. J., Galef, B. G., Jr. & Mason, J. R. (1988). At biologically significant concentrations, carbon disulfide both attracts mice and increases their consumption of bait. *Journal of Wildlife Management, 52,* 502-507.

Galef, B. G., Jr. (1988). Communication of information concerning distant diets in a social, central-place foraging species: *Rattus norvegicus*. In *Social learning: Psychological and biologcial perspectives.* T. R. Zentall and B. G. Galef, Jr. (Eds.), Hillsdale, N. J: Erlbaum Associates, pp. 3-28.

Galef, B. G., Jr. (1989a). An adaptationist perspective on social learning, social feeding and social foraging in Norway rats. In *Contemporary issues in comparative psychology.* D. A. Dewsbury (Ed.), Sunderland, MA: Sinauer Associates, pp. 55-79.

Galef, B. G., Jr. (1989b). Enduring social enhancement of rats' preferences for the palatable and the piquant. *Appetite, 13,* 81-92.

Galef, B. G., Jr., Kennett, D. J. & Stein, M. (1985). Demonstrator influence on observer diet preference: Effects of simple exposure and the presence of a demonstrator. *Animal Learning & Behavior, 13,* 25-30.

Galef, B. G., Jr., Mason, J. R., Preti, G. & Bean, N. J. (1988). Carbon disulfide: A semiochemical mediating socially-induced diet choice in rats. *Physiology and Behavior, 42,* 119-124.

Galef, B. G., Jr. & Stein, M. (1985). Demonstrator influence on observer diet preference: Analyses of critical social interactions and olfactory signals. *Animal Learning & Behavior, 13,* 31-38.

Galef, B. G., Jr. & Wigmore, S. W. (1983). Transfer of information concerning distant foods: A laboratory investigation of the 'information-centre' hypothesis. *Animal Behaviour, 31,* 748-758.

Galef, B. G., Jr., Whitmore, S. W. & Kennett, D. J. (1983). A failure to find socially-mediated taste-aversion learning in Norway rats *(Rattus norvegicus). Journal of Comparative Psychology, 97,* 358-363.

Mason, J. R., Bean, N. J. & Galef, B. G., Jr. (1989). Attractiveness of carbon disulfide to wild Norway rats. *Proceedings of the Thirteenth Vertebrate Pest Conference A.C.* Crabb and R. E. Marsh (Eds.), Davis, CA: University of California Press, 95-97.

Nowak, R. M. & Paradiso, J. L. (1983). *Walker's mammals of the world.* Vol 2, 4th Edition. Baltimore, MD: Johns Hopkins University Press.

Posadas-Andrews, & Roper, T. J. (1983). Social transmission of food preferences in adult rats. *Animal Behaviour, 31,* 265-271.

Simpson, G. G. (1961). *Principles of animal taxonomy.* New York: Columbia University Press.

Ward, P. & Zahavi, A. (1973). The importance of certain assemblages of birds as "information centres" for food finding. *Ibis, 115,* 517-534.

The International Journal of Comparative Psychology, Vol. 2, No. 4, Summer 1989

MOTIVATIONAL VARIATIONS IN THE SINGING BEHAVIOR OF A SIAMANG PAIR *(HYLOBATES SYNDACTYLUS)*

M. M. Haraway and E. G. Maples
Northeast Louisiana University

ABSTRACT: Several experiments have shown that engaging in territorial singing is an appetitive and reinforcing activity in gibbons. The present study examined whether the strength of this behavior would vary with changes in motivational conditions in the same manner as does the strength of the consummatory behavior associated with other reinforcers. The subjects were a fully accommodated pair of siamangs. Following baseline. ($\bar{X}$ duration = 34.33 min), song-bout durations were observed under low motivation ($\bar{X}$ = 20.33 min), then high motivation ($\bar{X}$ = 36.16 min), then low ($\bar{X}$ = 22.67 min), then high ($\bar{X}$ = 32.50 min). Six song bouts were observed under each condition. In the high motivation condition, 5-6 days intervened between song bouts; in the low motivation condition, song bouts were separated by 2 days. Each change in motivation was accompanied by a significant change in song-bout duration (Mann-Whitney *U* tests; *p*'s $< .01$). Findings are related to a general conception of species-typical behavior as a source of reinforcement.

The gibbons are small arboreal apes inhabiting the climax forests of Southeast Asia. All species of gibbons live in monogamous family groups on relatively fixed territories, and all perform elaborate, species-specific vocal displays, or songs (Carpenter, 1940; Chivers, 1972, 1974, 1976; Ellefson, 1968, 1974; Haimoff, 1981, 1984; Gittens, 1978; Marshall & Marshall, 1976; Marshall & Sugardjito, 1986; Tenaza, 1976; Whitten, 1982). Gibbon songs are assumed to function as a means of advertising territorial possession and of development and maintenance of cohesion within the family group (Carpenter, 1940; Haimoff, 1984; Marshall & Marshall, 1976).

Special acknowledgment is due to Amy Shadoin, Ronnie Santana, Charles Seltzer, Jenna Stewart, Donna Stafford, Lynndi Maddox, Roy Douget, Todd Stearns, Helen Bramlett, Amanda King, Cindy Smith, Marty Dunham, and Paul Rushing, all of whom assisted in the collection of data for this study.

Reprint requests and all correspondence should be sent to Dr. Ernest G. Maples, Psychology Department, Northeast Louisiana University, Monroe LA 72109.

Experiments with three species of gibbons (*Hylobates syndactylus*, *Hylobates agilis*, and *Hylobates muelleri*) suggest that their territorial singing, particularly within the natural context of accompanying vocal responses of family members and neighbors, is an appetitive behavior in these animals. In each of the experiments (Haraway, Maples, & Tolson, 1981, 1988; Maples & Haraway, 1982), the duration of the subject's vocal responding increased when tape-recorded accompaniment was made contingent on subject-vocalizations, decreased when this contingency was revoked, and increased again when the contingency was re-established. In each case, the extinction effect occurred only following a period of persistent high strength in the subject's vocal response, that is, only following resistance to extinction. In the last of these three experiments, it was found that playback of recorded vocalization also served to reinforce locomotor and station-keeping responses, in addition to the subject's *vocal* responding.

What these experiments show, specifically, is that appropriate playback stimulation serves as a positive reinforcer of responses that produce it, particularly if these are singing responses. One interpretation of these findings is that singing behavior in gibbons has reinforcement value, generally, and that its reinforcement value is *enhanced* by the occurrence of appropriate accompanying vocalizations.

Given these demonstrations of the reinforcement value of singing in gibbons, it is reasonable to ask whether the strength of such singing behavior varies with motivational conditions in the same manner as does the strength of consummatory behaviors that are associated with other sources of reinforcement. For example, we know that food deprivation increases the amount of eating behavior which occurs once food becomes available (e.g., Dufort & Wright, 1962; Horenstein, 1951); and, of course, a similar relationship exists between water deprivation and drinking behavior (Adolf, 1950; Siegel, 1947; Stellar & Hill, 1952). The present experiment, then, related the strength of the subjects' singing response to a systematic variation in the length of the interval between successive song bouts. The authors wished, specifically, to determine whether the subjects would perform longer song bouts under conditions when their song bouts were infrequently performed than under conditions when their song bouts were frequently performed.

The subjects of this experiment were a pair of siamang gibbons who, under normal circumstances, were always housed together. There was no readily available way to impose a deprivation on the singing behavior of these subjects, since they could effectively engage in such behavior almost at any time. However, because the male member of this pair would initiate a song bout almost immediately in response to presentation of recorded siamang vocalization; it was possible to arrange for the subjects to vocalize *more frequently* than at their normal interval of song

bout initiation. In this way, it was possible to generate a comparison of the relative strength of the subjects' singing behavior under contrasting motivational conditions.

METHOD

Subjects

The subjects, again, were a mated pair of siamang gibbons. The male, approximately 19 years of age, was wild-caught at an early age, and had lived at the Louisiana Purchase Gardens and Zoo in Monroe, Louisiana for the past 18 years. His previous mate had died, and he had lived alone for a period of 12 years. The female, 7½ years of age, had lived with her own parental group at the National Zoological Park in Washington, D.C. until her arrival at Louisiana Purchase Gardens and Zoo. The pair had been living together for approximately 18 months at the beginning of the experiment and were housed indoors in adjoining and connecting cages, each measuring 10 m x 4 m x 4 m high. During the period of this experiment, the animals remained in the same cage together at almost all times. Coordinated performance of the species great call had been achieved by this pair by approximately 12 weeks following their introduction to one another (Maples, Haraway, & Hutto, 1989). Copulation was first observed in the pair at approximately 10 months after their introduction.

PROCEDURE

On each experimental day, experimenters were present at the zoo from approximately 8:30 a.m. to 11:00 a.m. to observe the subjects' singing behavior. All song bouts that occurred during this period of the morning were recorded on audio tape, and the duration of each song bout was determined. In addition, zookeepers monitored the occurrence of singing behavior by the subjects at other times of the day. Zookeeper's reports were considered in determining the timing of procedural events during the controlled (nonbaseline) portions of the experiment, although these bouts, which were relatively few in number, were not audio-recorded and were not timed. The data for this study consist of 27 song bouts recorded between January, 1987, and October, 1987.

The experiment began with the recording of three song bouts under baseline conditions. Experimenters simply appeared at the zoo every morning and recorded any song bouts that occurred.

Next, the duration of the subjects' song bout was observed under a condition of relatively low motivation. For the purpose of this experi-

ment, degree of motivation was defined by the number of days that had passed since the subjects' most recent song bout. Extensive observation of these subjects had shown that their usual rate of song initiation was one song bout approximately every 5 days. During the low motivation condition, a song bout was stimulated by our recorded vocalization every other day until six song bouts had been recorded. We may note, here, that while the alternate-day frequency of song initiation was a relatively high rate of occurrence for this particular pair of siamangs, it is not a very high rate of song occurrence for this species, generally considered (Chivers, 1976). The male typically responded to recorded stimulation almost immediately, and the stimulation was terminated following the male's second vocal phrase.

After six song bouts were observed under the low motivation condition, song bout duration was observed under a condition of relatively high motivation. During this phase of the experiment, song bouts were stimulated only if six days had passed since the previous song bout. In addition, any song bouts which occurred on the fifth day following the previous song bout also were counted for data analysis. Two such song bouts occurred during the experiment. If a song bout occurred, or was reported by zookeepers, closer in time than 5 days from

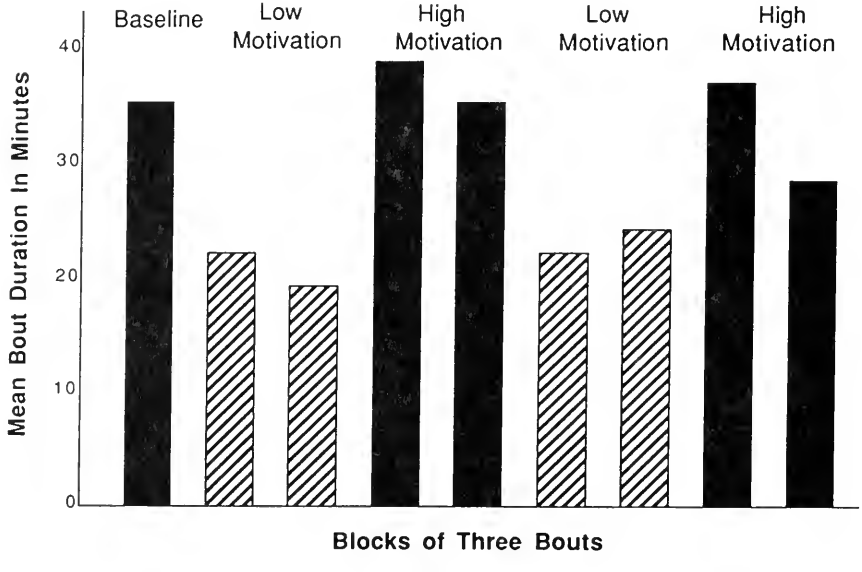

FIGURE 1

Mean song-bout duration under the various motivational conditions.

the preceding song bout, that song bout was excluded from consideration, and the experimenters waited 6 more days before stimulating the next song bout. During the high motivation condition, then, the interval between successive song bouts was approximately three times what it was during the low motivation condition. Finally, six additional song bouts were observed under the low motivation condition, and then six more bouts were observed under the high motivation condition.

RESULTS

It can be seen in figure 1 that song bout duration varied systematically with the variations in motivational conditions employed in this study. Mean song-bout duration changed from 34.33 min during baseline to 20.33 min under low motivation, then to 36.16 min during high motivation, then to 22.67 min during low motivation, and finally to 32.50 min during high motivation. The overall $\bar{X}$ duration under low motivation was 21.50 min, and under the combined conditions of baseline and high motivation, $\bar{X}$ was 34.33.

Mann-Whitney *U*-Tests were used to compare song-bout durations observed before and after each of these changes in motivational condition. Each change was accompanied by a significant variation in song bout duration; *U*-values ranged from 0 to 3, and all probabilities were less than .01, two-tailed.

The authors wish to emphasize that formal inferences from these statistical comparisons are limited to the behavior of these two subjects. Within the parameters of the present experiment, however, we may conclude that these subjects perform longer song bouts under conditions of high motivation than under conditions of low motivation.

DISCUSSION

The findings of the present study demonstrate that singing behavior in a pair of siamang gibbons varies systematically with motivational conditions in the same manner as do consummatory responses associated with such traditional reinforcers as food and water. Earlier studies (Haraway, et al., 1981, 1988; Maples & Haraway, 1982) have demonstrated the reinforcing value of stimuli (tape-recorded playbacks of gibbon songs) which provide a context for the occurrence of gibbon song. Taken together, these studies provide evidence of a functional similarity between singing behavior in gibbons and traditional and archetypical sources of appetitive reinforcement. Selective benefits potentially gained by the evolutional investment of gibbon song with reinforcement value were discussed in an earlier paper (Haraway, et al., 1988).

Glickman and Schiff (1967) proposed that species-typical behaviors, generally, constitute an important source of reinforcement. They suggested that the physiological substrates which underlie reinforcement functions may have evolved originally as means of facilitating the appropriate occurrence of species-typical behaviors. A survey of the literature on reinforcement in animal behavior reveals a surprisingly large number of research findings which may be interpreted as support for Glickman and Schiff's hypothesis.

A number of studies have achieved formal demonstration of reinforcement effects by using what may be regarded as species-typical behaviors in the role of reinforcers. These behaviors have included running behavior in rats and mice (Hill, 1956; Kagan & Berkun, 1954; Kavanau, 1966), sand-digging in mice (King & Weisman, 1964), social play in juvenile rats (Crowder & Hutto, 1988; Humphreys & Einon, 1981; Panksepp, Siviy, & Normansell, 1984), and exploratory behavior in a variety of species (Butler, 1953; Butler & Harlow, 1954; Butler & Wollpy, 1963; Chapman & Levy, 1957; Montgomery, 1954; Montgomery & Segall, 1955; Mote & Finger, 1942; Nissen, 1930; Schneider & Gross, 1965). Obviously, the great number of studies illustrating the reinforcement value of eating food, drinking water, and engaging in sexual behavior could be readily interpreted in the same manner.

In addition, a number of studies have demonstrated the reinforcing effectiveness of stimuli which establish an appropriate context for the occurrence of species-typical behaviors. Stimuli that have been shown effective in this fashion include an "imprinted" object, with chicks (Hoffman, Searle, Toffey, & Kozma, 1966); a rival male, with nesting sticklebacks (Sevenster, 1968), Siamese fighting fish (Melvin, 1985; Thompson, 1963), and domestic roosters (Thompson, 1964); and the song of another male, with chaffinches (Stevenson, 1967). Of course, the previously mentioned demonstrations of the reinforcement value of recorded gibbon song (Haraway, et al., 1981, 1988; Maples & Haraway, 1982) also fit into this category.

As we have seen, much evidence exists to support the generality that many species-typical behaviors are reinforcing in themselves, with the capability to serve as activity reinforcers for other behaviors which enable their occurrence. Much empirical work remains to be done in determining the proper parameters of this generality. It may be that few, if any, simple reflexes are reinforcing in this manner; and many species-typical behaviors involving defense from predators and from noxious stimuli may be sources of negative rather than positive reinforcement (Glickman & Schiff, 1967; Siminov, 1983). Whatever appropriate parameters are eventually established, species-typical behaviors appear to be a potent source of reinforcement in animals—and a source that often has been ignored.

REFERENCES

Adolf, E. F. (1950). Thirst and its inhibition in the stomach. *American Journal of Physiology, 161*, 374-386.

Butler, R. A. (1953). Discrimination learning by rhesus monkeys to visual-exploration motivation . *Journal of Comparative and Physiological Psychology, 46*, 95-98.

Butler, R. A. & Harlow, H. F. (1954). Persistence of visual exploration motivation. *Journal of Comparative and Physiological Psychology, 47*, 257-263.

Butler, R. A. & Wollpy, J. H. (1963). Visual attention in the rhesus monkey. *Journal of Comparative and Physiological Psychology, 56*, 324-328.

Carpenter, C. R. (1940). A field study of the behavior and social relations of the gibbon *(Hylobates lar). Comparative Psychology Monographs, 16*, 1-212.

Chapman, R. M. & Levy, N. (1957). Hunger drive and reinforcing effect of novel stimuli. *Journal of Comparative and Physiological Psychology, 50*, 233-238.

Chivers, D. J. (1972). The siamang and the gibbon in the Malay Peninsula. In D. Rumbaugh (Ed.), *Gibbon and siamang* (Vol. 1, pp. 103-135). Basel: Karger.

Chivers, D. J. (1974). The siamang of Malaya: A field study in a tropical rain forest. *Contributions to Primatology, 4*, 1-335.

Chivers, D. J. (1976). Communication within and between family groups of siamang *(Symphalangus syndactylus). Behavior, 57*, 115-135.

Crowder, W. F., & Hutto, C. W. (1988). *Social play reinforcement with young albino rats: A new place conditioning method.* Unpublished manuscript, The University of Mississippi.

Dufort, R. H., & Wright, J. H. (1962). Food intake as a function of duration of food deprivation. *Journal of Psychology, 53*, 465-468.

Ellefson, J. O. (1968). Territorial behaviour in the common white-handed gibbon *(Hylobates lar)*. In P. Jay, (Ed.) *Primates: Studies in adaptation and variability* (pp. 180-199). New York: Holt, Rinehart and Winston.

Ellefson, J. O. (1974). A natural history of white-handed gibbons in the Malayan peninsula. In D. Rumbaugh (Ed.), *Gibbon and siamang* (Vol. 3, pp. 1-136). Basel: Karger.

Gitten, S. P. (1978). Hark! The beautiful song of the gibbon. *New Scientist, 80*, 832-834.

Glickman, S. & Schiff, B. (1967). A biological theory of reinforcement. *Psychological Review, 74*, 81-109.

Haimoff, E. H. (1981). Video analysis of siamang *(Hylobates syndactylus)* songs. *Behaviour, 76*, 128-151.

Haimoff, E. H. (1984). Acoustic and organizational features of gibbon songs. In H. Preuschoft, D. Chivers, W. Brockelman, and N. Creel, (Eds.), *The lesser apes: Evolutionary and behavioral biology* (pp. 333-353). Edinburgh: Edinburgh University Press.

Haraway, M., Maples, E., & Tolson, S. (1981). Taped vocalization as a reinforcer of vocal behavior in a siamang gibbon *(Symphalangus syndactylus). Psychological Reports, 49*, 995-999.

Haraway, M., Maples, E. & Tolson, S. (1988). Responsiveness of a male Mueller's gibbon to his own species song, that of a Lar gibbon, and a synthetic song of similar frequency. *Zoo Biology, 7*, 35-46.

Hill, W. F. (1956). Activity as an autonomous drive. *Journal of Comparative and Physiological Psychology, 49*, 15-19.

Hoffman, H. S., Searle, J. L., Toffey, S., & Kozma, F. (1966). Behavioral control by an imprinted stimulus. *Journal of the Experimental Analysis of Behavior, 9*, 177-189.

Horenstein, B. R. (1951). Performance of conditioned responses as a function of strength of hunger drive. *Journal of Comparative and Physiological Psychology, 44*, 210-224.

Humphreys, A. P., & Einon, D. F. (1981). Play as a reinforcer for maze learning in juvenile rats. *Animal Behaviour, 29*, 259-270.

Kagan, J. & Berkun, M. (1954). The reward value of running activity. *Journal of Comparative and Physiological Psychology, 47*, 108.

Kavanau, J. L. (1966). Wheel running preference of mice. *Zeitschrift Fur Tierpsychologie, 23*, 858-866.

King, J. Q. & Weisman, R. G. (1964). Sand digging contingent upon bar pressing in deermice *(Peromyscus). Animal Behaviour, 12,* 446-450.
Maples, E. & Haraway, M. (1982). Taped vocalization as a reinforcer of vocal behavior in a female agile gibbon *(Hylobates agilis). Psychological Reports, 51,* 11-18.
Maples, E. G., Haraway, M. M., & Hutto, C. (1989). Development of coordinated singing in a newly formed siamang pair *(Hylobates syndactylus). Zoo Biology, 8,* 367-368.
Marshall, J. T. & Marshall, E. R. (1976). Gibbons and their territorial songs. *Science, 193,* 235-237.
Marshall, J. T. & Sugardjito, J. (1986). Gibbon Systematics. In D. Swindler and J. Erwin (Eds.). *Comparative Primate Biology: Systematics, Evolution, and Anatomy* (Vol 1, pp. 137-185). New York: Alan R. Liss, Inc.
Melvin, K. B. (1985). Attack display as a reinforcer in *Betta splendens. Bulletin of the Psychonomic Society, 23,* 350-352.
Montgomery, K. C. (1954). The role of exploratory drive in learning. *Journal of Comparative and Physiological Psychology, 47,* 60-64.
Montgomery, K. C. & Segall, M. (1955). Discrimination learning based upon the exploratory drive. *Journal of Comparative and Physiological Pyschology, 48,* 225-228.
Mote, F. A. & Finger, F. W. (1942). Exploratory drive and secondary reinforcement in the acquisition and extinction of a simple running response. *Journal of Experimental Psychology, 31,* 57-68.
Nissen, H. W. (1930). A study of exploratory behavior in the white rat by means of the obstruction method. *Journal of Genetic Psychology, 37,* 361-376.
Panksepp, J., Siviy, S., & Normansell, L. (1984). The psychobiology of play: Theoretical and methodological perspectives. *Neuroscience and Biobehavioral Reviews, 8,* 465-492.
Schneider, G. E. & Gross, C. G. (1965). Curiosity in the hamster. *Journal of Comparative and Physiological Psychology, 59,* 150-152.
Sevenster, P. (1968). Motivation and learning in sticklebacks. In P. Ingle, (Ed.), *The central nervous system and fish behavior* (pp. 233-245). Chicago: University of Chicago Press.
Siegel, P. S. (1947). The relationship between voluntary water intake body weight loss, and number of hours of water privation in the rat. *Journal of Comparative and Physiological Psychology, 40,* 231-238.
Siminov, P. V. (1983). Avoidance reactions to tape-recorded pain cry in rats. *Neuroscience and Behavior Physiology, 13,* 87-89.
Stellar, E. & Hill, J. H. (1952). The rat's rate of drinking as a function of water deprivation. *Journal of Comparative and Physiological Psychology, 45,* 96-102.
Stevenson, J. G. (1967). Reinforcing effects of chaffinch song. *Animal Behaviour, 15,* 427-432.
Tenaza, R. R. (1976). Song, choruses and countersigning of Kloss' gibbons *(Hylobates klossi)* in Siberut Island, Indonesia. *Zeitschrift fur Tierpsychologie, 40,* 37-52.
Thompson, T. I. (1963). Visual reinforcement in Siamese fighting fish. *Science, 141,* 55-57.
Thompson, T. I. (1964). Visual reinforcement in fighting cocks. *Journal of the Experimental Analysis of Behavior, 7,* 45-49.
Whitten, A. J. (1982). The ecology of singing in Kloss' gibbons *(Hylobates klossi)* on Siberut Island, Indonesia. *International Journal of Primatology, 3,* 33-51.

INTERNATIONAL JOURNAL OF COMPARATIVE PSYCHOLOGY

Volume 2, Number 1

INTERNATIONAL JOURNAL OF COMPARATIVE PSYCHOLOGY

Volume 2, Number 2

Articles

INTERNATIONAL JOURNAL OF COMPARATIVE PSYCHOLOGY

Volume 2, Number 3

Articles

INTERNATIONAL JOURNAL OF COMPARATIVE PSYCHOLOGY

Volume 2, Number 4

ARTICLES

Instructions to Authors

All manuscripts are reviewed by two readers other than the Editors. Authors should send three copies of their manuscripts to the National Editor of their country. If there is no editor, the paper should be sent to the Editor (Tobach). See inside front cover for editors' addresses. The manuscript should have a cover page with the full title, the author(s) name(s) and address(es) and should indicate the person with whom the Journal may correspond. The abstract should be on a separate page. All articles are published in English; the author(s) may also submit an abstract in another language which does not require non-Roman type. Author(s) are responsible for the accuracy of the non-English abstract. Acknowledgements should appear on a separate page and carry the title of the article and the author(s) name(s). If the author(s) prefer(s) anonymity in the review process, the manuscript, abstracts and illustrative material should carry only the title of the article. The cover sheet and acknowledgements will not be sent to the reviewers in this instance.

GENERAL REQUIREMENTS: Logical organization is essential. While headings help to structure the content, titles and headings within the manuscript should be as short as possible. Tables and figures should not be redundant. Illustrative material should be self-explanatory and used sparingly. Tables and figures must be in camera-ready condition and include captions. Only items cited in manuscripts should be listed as references. Page numbers must be provided for direct quotations. Footnotes should not be used except in special circumstances and with permission of the Editor.

MANUSCRIPTS should be double spaced, the original on white bond paper, with margins of 3cm on all four edges and approximately 26 lines to the page. Manuscripts longer than 20 typed pages (not counting figures, tables and references) will only be considered in unusual circumstances. The style manual of the American Psychological Association (3rd Edition, 1983) is to be followed. If the manual is not available, authors may write to the National Editor or the Journal Editors (Tobach or Thompson) for a style sheet.

REFERENCES should be listed on a separate page and referred to in the text by author(s) and year of publication as in the Publication Manual of the American Psychological Association.